Guadalupe River Watershed Mercury TMDL: 2016-2017 Progress Report on Methylmercury Production and Control Measures

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1 Executive Summary

The Guadalupe River Watershed in the south San Francisco Bay is contaminated with mercury derived largely from the New Almaden Mining District (Mining District). The Mining District, which remained in production from 1846 until 1975, produced about 38.4 million kilograms of mercury. Mercury was extracted from cinnabar on-site, yielding vast quantities of waste rock, calcines, and tailings. Miners disposed of these mercury-rich byproducts in the waterways of the upper Guadalupe River Watershed, resulting in elevated mercury concentrations from the Mining District to the San Francisco Bay. In lentic water bodies experiencing seasonal periods of anoxia, mercury can be microbially converted to bioavailable monomethylmercury (methylmercury), a potent neurotoxin which biomagnifies in the food chain and presents sizeable risks to birds and humans that consume fish. The San Francisco Bay Regional Water Quality Control Board (Regional Board) addressed mercury pollution in the Guadalupe River Watershed by amending the Water Quality Control Plan for the San Francisco Bay Basin (Basin Plan) in 2008 to establish the Guadalupe River Watershed Mercury TMDL (TMDL).

To remain in compliance with the implementation actions set forth by the TMDL, the District is required to complete studies of methylmercury and bioaccumulation control measures, and to implement effective controls. These studies include a comparative analysis of the lakes and reservoirs affected by mercury mining, an effectiveness evaluation of hypolimnetic oxygenation and circulation in reducing methylmercury production and bioaccumulation, and calculation of inorganic and organic mercury loads from points of discharge. The District is required to demonstrate progress in these studies by reporting to the Regional Board by December 31 of odd years. These studies include findings specific to the mercury-impaired reservoirs affected by the TMDL (Almaden, Calero, and Guadalupe reservoirs, and Almaden Lake), as well as Stevens Creek Reservoir, which is located outside of the Guadalupe River Watershed and serves as an additional reference site.

District Staff collected monthly (during mixed conditions) to bi-monthly (during thermal stratification) water quality profiles and grab samples of mercury species and nutrients from different points in the water columns of the reservoirs, as well as water quality measurements and samples of mercury species at gaged outlet structures. Fish samples were collected twice-annually during the spring (March to April) and summer (August to September) for body burden mercury analysis. Reservoir hypolimnetic oxygenation systems were operated nearly-continuously during stratified conditions occurring from approximately March to October in Almaden, Calero, Guadalupe, and Stevens Creek reservoirs. Four solar-powered hypolimnetic circulators (Solar Bees) were operated in Almaden Lake. Staff used parametric and non-parametric analysis of variance, statistical methods for censored data, and multiple linear regression to evaluate effectiveness of the treatment systems in improving water quality and reducing bioaccumulation in the reservoirs and lake.

Inorganic mercury concentrations were highest during the wet season, suggesting mobilization and transport during high-intensity flow events. Methylmercury production occurred predominantly in the hypolimnia (bottom layer) of the reservoirs and lakes under low-oxygen conditions during thermal stratification. Methylmercury production coincided with hypolimnetic sulfate depletion, implicating anaerobic sulfate-reducing bacteria as the primary methylators of inorganic mercury. Coincidingly, anaerobic conditions facilitated internal loading of ammonia and phosphorus from bottom-sediments. Methylation efficiency was found to increase with inorganic mercury concentration and nutrient enrichment. Aerobic decomposition of algal biomass depletes oxygen in bottom-waters, creating favorable conditions for the proliferation of anaerobic bacteria. However, bioaccumulation of mercury was reduced in more eutrophic water bodies, likely the effect of bloom dilution, which reduces methylmercury concentrations in phytoplankton by spreading it among more biomass.

During operation of the hypolimnetic oxygenation systems, dissolved oxygen concentrations improved significantly in the hypolimnia of all reservoirs except Calero Reservoir. Sulfate concentrations increased significantly in all reservoirs except Almaden Reservoir, suggesting attenuation of microbial sulfate reduction. Correspondingly, hypolimnetic methylmercury concentrations decreased significantly in all reservoirs except Almaden, rarely exceeding the TMDL water quality allocation for reservoirs of 1.5 ng/L. However, methylmercury concentrations in the upper water column (photic zone) either increased or were unchanged. This is concerning, because the largest biomagnification (100,000x increase) of methylmercury occurs in the photic zone between the water column and phytoplankton. In some cases, hypolimnetic total phosphorus and ammonia concentrations were reduced during system operation. However, epilimnetic total phosphorus concentrations increased in all reservoirs during oxygenation. Increased phosphorus concentrations in the photic zone may be responsible for increased chlorophyll *a* concentrations observed during system operation. While increased algal biomass may provide additional organic detritus to compound hypolimnetic anoxia and increase methylation efficiency, it may also decrease mercury concentrations in biota through bloom dilution. High epilimnetic methylmercury and total phosphorus concentrations during system operation may be the result of advective transport of profundal compounds with the bubble plumes as bubbles rise to the surface. Alternatively, these compounds may be produced in littoral or marginal sediments in the water columns of the reservoirs. On a two-week monitoring interval, data has not documented a pulse of methylmercury or nutrients into the photic zone following fall turnover (lake mixing), as described in other studies. This may be due to rapid algal uptake following turnover, or the reservoirs' outlet configurations, which discharge water downstream through hypolimnetic withdrawal, removing profundal compounds from the reservoirs.

Only Guadalupe Reservoir exhibited a significant declining trend in fish tissue mercury concentrations during hypolimnetic oxygenation, but mercury concentrations continue to far exceed EPA guidelines for fish consumption. In Stevens Creek Reservoir, fish tissue mercury concentrations increased during oxygenation. The ineffectiveness of the oxygenation systems in reducing fish tissue mercury concentrations is likely a result of high methylmercury concentrations measured in the photic zones of the reservoirs during system operation.

Almaden Lake's solar circulators did not significantly increase hypolimnetic dissolved oxygen concentrations, and its hypolimnion remained anoxic for much of the dry season. Despite this, hypolimnetic sulfate concentrations increased and methylmercury concentrations decreased. Methylmercury concentrations frequently exceeded 1.5 ng/L throughout the water column at the lake's main quarry pit. Though internal nutrient loading appeared to decrease, as evident by reductions in hypolimnetic total phosphorus and ammonia concentrations, algal productivity increased following the installation of the circulators. Though dissolved oxygen concentrations were unaffected by circulation, it is plausible that redox conditions were improved sufficiently to delay reducing processes. Additionally, mechanical dilution of profundal compounds throughout the water column may affect the perceived water quality changes. Please see AECOM's *Final Guadalupe River Coordinated Monitoring Program 5-Year Report* for discussions of trends in fish tissue mercury concentrations in Almaden Lake.

The District is committed to continuing its voluntary studies of methylmercury production and control measures, and will notify the Regional Board should any capital projects occur that may impede monitoring efforts. The District intends to continue operation of the methylmercury control systems, and does not plan to modify operational procedures during the next two-year reporting period.

The District provides the following recommendations for current and future studies of methylmercury control systems:

Current District Study

- The District requests the elimination of Sampling Sites 2-5 at Almaden Lake. Site one, containing the first solar circulator installed (included in this report), and the inlet and outlet sites are sufficient for assessing treatment system effectiveness.

- The District requests to discontinue manganese and iron sampling in reservoirs. No data were collected in Almaden Lake, Almaden Reservoir, Guadalupe Reservoir, and Stevens Creek Reservoir prior to the installation of the treatment systems, so there is no baseline to compare to. Additionally, this report demonstrates these analytes as poor predictors of redox potential.

- The District requests to discontinue outlet sampling at Almaden, Calero, and Stevens Creek reservoirs, because outlet samples were found to be statistically indistinguishable from samples collected in their hypolimnia.

- The District considers Special Study 1 to be complete. If the Regional Board is satisfied with the results of the comparative study presented in this document, which incorporate 10 years of monitoring data, the District requests to discontinue monitoring of the following analytes: Ammonia, Total Phosphorus, Nitrate, and Nitrite. The TMDL does not require monitoring of nutrient cycling, or effectiveness evaluation of treatment systems regarding internal nutrient loading.

Following approval from the Regional Board, the District will produce an updated plan describing future monitoring activities.

Statewide Mercury Control Program

- Consider dry-season methylmercury concentrations present in the photic zone when evaluating treatment system effectiveness. Fish foraging and metabolism decreases during the winter months, so dietary exposure to methylmercury is most significant during the dry season.
- Line diffuser systems may not be ideal systems for large or weakly-stratified reservoirs like Calero Reservoir. Oxygen retention appears to be more efficient in smaller reservoirs with high relative depths.
- Consider the effects of outlet structure on profundal compounds available to the photic zone during turnover. Different outlet structure elevations may impact compound movement during and after reservoir turnover. Additional studies with frequent monitoring during reservoir turnover may be needed to capture these effects. The District may conduct special studies related to the effects of variable outlet configurations in the future, as Calero Reservoir's outlet structure may be replaced or modified as part of the Calero Dam Seismic Retrofit Project.
- The variable foraging patterns of young largemouth bass lead to high variance in mercury concentrations, perhaps making them a poor short-term indicator of treatment system effectiveness. Less predatory fish should be used whenever possible in future studies mandated by the State Water Resources Control Board.

2 Acknowledgments

The Santa Clara Valley Water District's studies of methylmercury production, bioaccumulation, and controls are funded by the *Safe, Clean Water and Natural Flood Protection Program*, a fifteen-year ballot measure passed by an overwhelming majority in November of 2012. The District is committed to protecting our environment and is working to reduce toxins, hazards, and contaminants in Santa Clara County waterways under Priority B of the Safe, Clean Water Program.

This project is a collaborative effort involving many internal and external contributors. I would like to thank Jennifer Castillo and Kirsten Struve of the District's Environmental Planning Unit for providing support and encouragement, as well as for overseeing this project. Roy Hays and Brett Calhoun, also of the Environmental Planning Unit, ensured continuous operation of the reservoir hypolimnetic oxygenation systems during this reporting period: a challenging task that is crucial to the quality of this study. Vitaliy Vaysfeld of the District's Software Services Unit developed the Environmental Monitoring Information Management System (EM-IMS), which contains many tools essential to data collection, storage, and analysis. Funding for implementing this database was provided by Priority D5 of the Safe, Clean Water Program, facilitated by Doug Titus of the Environmental Mitigation and Monitoring Unit. Clayton Leal, Fisheries Biologist of the same unit, managed all fish collections and provided valuable expertise and insights related to fish population dynamics and bioaccumulation. The following current or former District staff assisted regularly with field sample collection: Meagan Beaver, Tiffany Chao, José De Guzman, Kendra Mann, Amanda Sauao, Spencer Seale, Rachel Smith, Billy Tu, and Elisabeth Wilkinson. The District's equipment management unit maintained all vehicles and vessels used to perform data collection.

I would like to thank our technical review panel, whose comments and recommendations greatly improved the quality of this report. Professor Marc Beutel of University of California, Merced offered expertise in the fields of limnology and methylmercury treatment systems. Dr. Darell Slotton of University of California, Davis provided review and comments related to fish tissue collection and analysis. Data scientist Dr. Dan Urban provided insights into the statistical methods used to infer trends and treatment system effectiveness. I would also like to thank the District's internal review panel.

3 Introduction

The Guadalupe River Watershed in Santa Clara County, California, is contaminated by the former New Almaden Mining District: North America's oldest and most productive mercury mine, and the fifth largest in the world. Though active mining halted by 1970, waste rock and contaminated sediments persist as sources of mercury to the watershed. The Santa Clara Valley Water District (District) manages four water bodies affected by historical mining operations: Almaden, Calero, and Guadalupe reservoirs, and Almaden Lake. In 1999, these water bodies were included on the U.S. Environmental Protection Agency's Clean Water Act 303(d) list as impaired for mercury. In 2008, the San Francisco Bay Regional Water Quality Control Board (Regional Board) adopted an amendment to the Water Quality Control Plan for the San Francisco Bay Basin, establishing contaminant allocations and implementation plans for mine and reservoir owners in the watershed. The District maintains compliance with the Guadalupe River Watershed Mercury Total Maximum Daily Load (TMDL) by conducting and reporting on the technical studies included herein.

Almaden, Calero, and Guadalupe reservoirs were constructed in the 1930s for water conservation. The three reservoirs are in the upper Guadalupe River Watershed, which drains to the San Francisco Bay (Appendix A.1). Mercury-laden sediments and waste material from the New Almaden Mining District contaminate Almaden and Guadalupe reservoirs, and Almaden Reservoir contaminates Calero Reservoir by water transfers through the Almaden-Calero Canal. Almaden Lake is the flooded remnant of an in- and off-stream gravel quarry that operated between 1950 and 1960. The lake is fed by Los Alamitos Creek, which receives discharge from Almaden and Calero Reservoirs. Its outlet is located 100 meters upstream of Los Alamitos Creek's confluence with Guadalupe Creek, which receives discharge from Guadalupe Reservoir. The confluence of Los Alamitos and Guadalupe creeks forms the main stem of the Guadalupe River, which flows to the southern San Francisco Bay (Appendix A.2). The lake is approximately 40 acres in area, with a maximum depth of 13 meters (43 feet). A proposed project intending to separate Almaden Lake from Los Alamitos Creek is in its planning phase.

The District is responsible for addressing the production and discharge of methylmercury in reservoirs contaminated by legacy mining waste. During the summer months, reservoirs and lakes commonly stratify into distinctive density layers. The warmer, less dense epilimnion floats atop the colder, denser hypolimnion. Decomposition of organic material depletes oxygen concentrations in the hypolimnion, creating low-oxygen conditions that can persist throughout the period of stratification. Anaerobic bacteria in bottom sediments convert divalent mercury to methylmercury [25], a highly-toxic organic molecule that can cause neurological damage and cardiovascular disease in humans. Methylmercury bioaccumulates in algae, prey, and predatory fish, increasing in concentration as it moves up the food chain. Fish in the watershed have been measured to contain mercury concentrations that exceed the United States Environmental Protection Agency's (USEPA) national criterion for protection of human health by over an order of magnitude.

A portion of this report evaluates the effectiveness of treatment options that intend to curtail the production of methylmercury by discouraging the establishment of seasonal hypoxia in the hypolimnia of lakes and reservoirs. Almaden Lake is equipped with four solar-powered hypolimnetic circulators intending to enhance hypolimnetic oxygen concentrations and avoid conditions conducive to the anaerobic conversion of mercury to methylmercury. Almaden, Calero, Guadalupe, and Stevens Creek reservoirs are currently equipped with hypolimnetic oxygenation systems, which generate and inject nearly-pure oxygen into the reservoir-bottoms through fine bubble diffusion. Stevens Creek Reservoir is located outside of the Guadalupe River Watershed, and serves as an additional reference site for evaluating treatment system effectiveness. However, because this reservoir receives treatment, it does not serve as a control.

Prior to the adoption of the Guadalupe River Watershed TMDL, the District initiated voluntary studies of aquatic methylmercury production, bioaccumulation, and control measures. Continued analyses include a comparative study of the physical, chemical, and biological differences of the reservoirs, evaluations of control system effectiveness in reducing methylmercury concentrations in the water column and fish tissue, and calculations of annual mercury loads at discharge points. The continuation of these studies is required to remain in compliance with the implementation and monitoring requirements set forth in the TMDL Staff Report [4]. As specified in the TMDL Staff Report (Section 9.4), this progress report from the District, detailing interim results of technical studies and treatment system evaluation, is due to the Regional Board by *December 31, 2017*. This report encompasses the reporting period of October 2015 through October 2017. The studies included herein are intended to address the monitoring requirements described in Table 9.1 of the TMDL Staff Report[1], including special studies, analysis of methylmercury treatment systems, and mercury loading at discharge points.

[1] https://www.waterboards.ca.gov/sanfranciscobay/water_issues/programs/TMDLs/guadalupe_river_mercury/C1_Guad_SR_Sep08.pdf

4 Baseline Study and Regulatory Background

In 1999, the Santa Clara Basin Watershed Management Initiative (SCBWMI) assembled the Guadalupe Mercury Work Group to assist with developing the technical basis for the TMDL. This group produced the *Work Plan to Develop and Implement a Total Maximum Daily Load (TMDL) For Water Bodies in the Guadalupe River Watershed Listed as Impaired Due to Mercury* (June 29, 2000) and the *Guadalupe River Mercury TMDL Workgroup's Recommended Interim Sampling and Monitoring Plan* (December 7, 2000).

In November of 2000, Santa Clara County voters passed a ballot measure creating the *Clean, Safe Creeks, and Natural Flood Protection Program*, which was implemented by the District. Under this 15-year measure, one million dollars per year was allocated to the improvement of impaired water bodies. One year of program funding was used to finance the baseline study and development of the conceptual model that form the foundations of the TMDL. SCBWMI selected Tetra Tech, Inc. (Tetra Tech) to perform these tasks.

In 2003, Tetra Tech published the *Preliminary Problem Statement and Synoptic Survey*, providing an overview of mercury contamination in the Guadalupe River watershed, and identifying major locations of mercury methylation. The key findings of these studies were the observation of extensive mining waste material in Los Alamitos Creek, and the implication of reservoirs and deep impoundments as crucial sites of methylmercury production. In 2004, Tetra Tech published the *Data Collection Plan for the Guadalupe River Watershed*. From 2003 to 2004, Tetra Tech conducted field data collection that provided estimates of wet-season mercury loads and baseline fish tissue mercury data throughout the watershed. In 2005, findings of these studies were referenced in the *Final Conceptual Model Report*, which additionally provided a watershed characterization, and conceptual model of mercury cycling and transformation. This document forms the basis of the *Guadalupe River Watershed Mercury TMDL Staff Report*.

In 2005, the District initiated a comprehensive monitoring program to improve understanding of mercury and nutrient cycling in the three impaired reservoirs (Almaden, Calero, Guadalupe) and Almaden Lake. These data confirmed that the seasonal production of methylmercury is associated with hypoxia in the hypolimnia of lentic water bodies. These data serve as baseline metrics to which data collected following the installation and operation of the control systems are compared.

In 2004 and 2005, the District prepared the *Stream Fish Tissue Collection and Analysis Report for Total Mercury Content in Lavinia Symmetricus* and the *Reservoir Fish Tissue Collection and Analysis Report for Total Mercury Content in Sport Fish*. These reports detailed mercury concentrations in fish collected in creeks and water bodies of the Guadalupe Watershed, providing body burden and age data used during the development of the TMDL. The Coordinated Monitoring Program (CMP) was established in 2011 following a California Water Code 13267 letter from the Regional Board to fulfill TMDL monitoring requirements common to all responsible parties (District, County of Santa Clara Parks and Recreation, Midpeninsula Regional Open Space District, Guadalupe Rubbish Disposal Company). The CMP is funded through a cost-share among the responsible parties, and is conducted on five-year cycles. Fish monitoring completed by the CMP in 2012, 2013, and 2016 further indicated high concentrations of mercury in watershed fish.

5 Study Descriptions

Section 9.4 of the TMDL Staff Report requires the District to demonstrate progress in reducing aqueous and fish tissue methylmercury concentrations in biennial reports to the Regional Board. These reports detail interim results of the following required studies.

5.1 Special Study 1: Comparative Study

Special Study 1 examines the physical, chemical, biological, and infrastructural differences between the four water bodies included in the TMDL. It seeks to elucidate the factors that may affect rates of methylmercury production and bioaccumulation. Section 9.10 of the TMDL Staff Report presents the following questions:

"How do the reservoirs and lakes in this watershed differ from one another? Factors to consider include, but are not limited to, area of connected wetlands, food web, water chemistry (phosphorus, pH, acid neutralizing capacity, and dissolved organic carbon), water level fluctuations, and infrastructure (outlet structure). Do outlet samples adequately represent hypolimnetic methylmercury concentrations for each reservoir? How significant are these differences?"

The District uses data collected prior to the installation of the treatment systems, and during winter periods of system inactivity, to investigate the similarities and differences of Almaden, Calero, and Guadalupe reservoirs, Almaden Lake, and the reference site.

5.2 Special Study 2: Treatment System Effectiveness

Special Study 2 addresses the following question posed in Section 9.10 of the TMDL Staff Report:

"Is it possible to increase the assimilative capacity for methylmercury in reservoirs and lakes? Is it feasible to do so? If it is feasible, does it result in attaining the fish tissue targets?"

The District addresses Special Study 2 by evaluating the efficacy of the control systems in reducing methylmercury concentrations in the water column and in fish in each water body. These systems may increase assimilative capacity by suppressing anoxic conditions that facilitate the bacterial conversion of mercury to methylmercury.

5.3 Mercury Loads at Discharge Points

Section 9.9 of the TMDL Staff Report requires the monitoring of mercury loads at the discharge points of reservoirs and lakes to assess progress in load reduction. These analyses integrate outlet grab sample and stream gauge data to estimate total mercury loading to downstream waters.

6 Monitoring Sites and Methods

6.1 Reservoir Monitoring Sites

Water quality profiles and samples were collected above the deepest portions of Almaden, Calero, Guadalupe, and Stevens Creek reservoirs near the outlet works (Appendices A.3 to A.6). Additional samples and water quality data were taken at reservoir outlet structures. Outlet data are used to calculate mercury discharges to downstream waters.

6.2 Lake Monitoring Sites

The bathymetry of Almaden Lake has been developed using echo-sounding equipment (Appendix A.7). The data indicates four distinct areas of significant depth, corresponding to the historical gravel quarry pits. Water quality profiles and samples were collected at five stations within the lake, adjacent to the solar circulators. Additional samples are collected at the inlet and outlet (Appendix A.8). Monitoring Site 1, and the inlet and outlet sites are referenced in this report.

6.3 Water Quality Monitoring

Reservoir depth profiles were collected using Hydrolab DS5 multiparameter sondes. Parameters measured include pH, temperature, oxidation-reduction potential, specific conductivity, dissolved oxygen, chlorophyll a, and phycocyanin. Profile data were logged at 0.25 to one-meter intervals throughout the water column. Water quality data were collected at reservoir outlets beginning in 2008. Various instruments have been used including Horiba U-10 multiparameter meters, Hanna Instruments HI 93414 turbidity meters, and YSI Professional Plus multi-parameter data collectors. Outlet data collected included pH, specific conductivity, turbidity, dissolved oxygen, and temperature. Water samples were collected using a Wildco alpha-type Van Dorn sampling device (2.2 liter) at discrete depths. Epilimnion samples were collected at a depth of two meters. Hypolimnion samples were collected approximately one meter above the lake or reservoir bottom. Prior to 2016, mid-depth samples were taken at three even intervals between the epilimnion and hypolimnion samples, termed epi-mid, mid, and mid-hyp. Following 2016, these samples were collected in the same manner when the reservoirs were not thermally stratified, and at the top, middle, and bottom of the thermocline during periods of stratification. The District made this modification to increase understanding of methylmercury distribution throughout the metalimnion during thermal stratification. Because the sampling method has changed, and to simplify interpretations by limiting results to the epilimnion and hypolimnion depths, we did not analyze mid-depth samples statistically. However, time-series plots of these data are available in Appendix C. Samples were dispensed using "Clean Hands-Dirty Hands" procedures of EPA Method 1669 into the containers described in Table 1.

Table 1: Sample Collection Bottles and Preservatives

Analyte	Container Material	Volume	Preservative
Ammonia (as N)	HDPE	500mL	Sulfuric Acid
Low-Level Mercury	Flourinated Polyethelene	250mL, double bagged	Unpreserved
Nitrate, Nitrite, Sulfate	HDPE	500mL	Unpreserved
Total Methylmercury	Flourinated Polyethelene	250mL, double bagged	Hydrochloric Acid
Total Mn, Total Fe	HDPE	250mL	Nitric Acid
Total Phosphorus	HDPE	250mL	Sulfuric Acid

6.4 Laboratory Analysis Methods

Table 2 describes the laboratory methods used for chemical analysis, as well as current reporting limits, below which measured values were considered "non-detects". Note that these reporting limits have changed over time, requiring the use of statistical methods for censored data when analyzing parameters with a significant percentage of non-detect values.

Table 2: Laboratory Analysis Methods

Analyte	Method	Current Reporting Limit
Ammonia (as N)	EPA 350.1	0.1 mg/L
Low-Level Mercury	EPA 1631 E	0.5 ng/L
Nitrate, Nitrite, Sulfate	EPA 300	1 mg/L
Total Iron	EPA 6010B	0.1 mg/L
Total Manganese	EPA 6010B	0.020 mg/L
Total Methylmercury	EPA 1630	0.05 ng/L
Total Phosphorus	SME 4500 P E	0.050 mg/L

6.5 Fish Tissue Monitoring

The District collects fish for body burden mercury analysis from Almaden, Calero, Guadalupe, and Stevens Creek reservoirs. The Coordinated Monitoring Program (CMP) collects fish from Almaden Lake and required stream sampling sites. Refer to CMP reports for descriptions of methods used[2]. Fish tissue data collected from Almaden Lake by the CMP is referenced in Special Study 1. Depending on reservoir water level, the District collected fish using either boat electrofishing or hook-and-line methods.

When boat electrofishing was used, fish were captured using a Smith-Root Model H electrofishing boat. Boat electrofishing samples the water column between the surface and approximately 15 feet of depth, depending on the conductivity and settings. Boat electrofishing possesses a sampling bias including the area that can be sampled, species catch ability, and netting efficiency. Only fish near shore or within the top of the water column can be collected, and reservoir conditions such as turbidity, aquatic vegetation, and water level limit sampling and netting ability. The pelagic tendency of forage fish makes them more susceptible to capture using boat electrofishing, so results may overestimate prey populations relative to predatory fish. Three to five sampling fetches were collected throughout the reservoirs. Fetches were defined as fifteen-minute passes of specific areas of shoreline, and the distance sampled depended on fish abundance and netting efficiency. Sampling was conducted at night to increase capture efficiency.

When low water levels prohibited the use of the electrofishing boat, samples were collected using hook-and-line sampling methods. Sampling was conducted from a 14-foot aluminum Jon Boat. Methods included open-water trolling along transects and stationary angling along shore margins. Hook-and-line sampling may present a bias toward larger fish, as gape size can limit catchability of smaller fish. Additionally, the sampling location and ability of the angler may confound the collection results. The primary goal of this sampling effort was to collect fish for the body burden analysis, so more emphasis was placed on collecting target fish than providing an estimate of fish assemblage or size distribution.

The body burden analysis targeted trophic level 3 and 4 fish, including (but not limited to) largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), and black crappie (*Pomoxis nigromaculatus*). All fish sampled fell within two size classes; 50 mm to 150 mm and 150 mm to 350 mm. Fish selected to be sacrificed were placed in individual zip-lock bag, labeled, and placed on ice for transport back to District facilities. The samples were then removed from the ice, processed (numbered, remeasured, weighed, and double-labeled), and placed in the freezer in preparation for transport to the laboratory.

6.6 Fish Collection Categories

During the 2016-2017 reporting period, fish were sampled twice-annually from March to April (spring sampling event) and August to September (summer sampling event). Three categories of fish were collected to respond to different study questions addressed by this report:

[2] `https://www.waterboards.ca.gov/sanfranciscobay/water_issues/programs/TMDLs/guadaluperivermercurytmdl.shtml`

Remediation Effectiveness Indicators (REIs) are samples designed to be sensitive measures of mercury exposure variability in space and time. In the Guadalupe River Watershed, based on recommendations from the Regional Board, we have chosen "age-1" largemouth bass ranging from 55 to 102 mm in length as the primary REIs [4]. Since largemouth bass spawn during the springtime, REI samples are collected during the summer sampling event to ensure adequate tissue mass for laboratory analysis. Largemouth bass within the REI size range collected during the springtime are likely to represent the previous year's cohort.

Target Fish (TL3A and TL3B) are defined as 50 to 350 mm trophic level 3 fish. These fish are collected to measure progress in attaining fish tissue objectives of 0.05 mg Hg/kg (wet weight) for 50-150 mm fish (TL3A), and 0.1 mg Hg/kg (wet weight) for 150-350 mm fish (TL3B). These allocations are intended to be protective of piscivorous birds. Thus, trophic level 3 target fish are collected just before or during the avian breeding season (spring sampling event), and during the summer sampling event.

Adult largemouth bass (TL4) range from 102 to 350 mm. Though these fish do not serve as targets or REIs, abundant historical data exists. Future adult largemouth bass samples will be collected during the spring sampling event only, representing the cohort of REI fish measured during the previous summer. This data will serve to determine bioaccumulation rates that occur during the wet season, as well as to minimize extrapolation in length-standardization.

6.7 Statistical Methods

All statistical tests were computed using the R programming language and environment for statistical computing. Mean comparisons were performed using a variety of methods. If the data were normally or near-normally distributed, or could be transformed to fit a normal or near-normal distribution, Analysis of Variance (ANOVA) was used. If the data could not be normalized, the Kruskal Wallis One-Way Analysis of Variance was used. For parameters that contained over five percent of non-detect results, mean comparisons and descriptive statistics were computed using the "cenfit" and "cendiff" functions of the NADA package for R. These tools model estimates based on empirical cumulative distribution functions (ECDF) for censored data using the Kaplan-Meier method, as described in Dennis Helsel's "Nondetects and Data Analysis; Statistics for censored environmental data," published in 2005 [45]. Trends in fish tissue mercury concentrations were conducted using multiple regression techniques. Mean comparisons of fish mercury concentrations were conducted using ANOVA to control for effects of length, collection season, and species.

Boxplots Boxplots are used in this report to present statistical data. The rectangle reflects the interquartile range (IQR) of the data, ranging from the 25th to 75th percentile. The upper edge of the rectangle reflects the 75th percentile, the value below which 75% of the data fall. The lower edge of the rectangle reflects the 25th percentile. The median value of the data is reflected by the horizontal line dividing the rectangle. The upper and lower whiskers display the upper and lower quartiles. Any value falling further than 1.5 IQRs below the first or above the third quartile is considered an extreme value, and is displayed as a point. For laboratory data, reporting limits are displayed using dashed red lines.

Depth Definitions Statistical comparisons are made using the terms "Epilimnion" and "Hypolimnion" to distinguish between surface and bottom waters. These terms represent upper (2 meters from surface) and bottom (1 meter from bottom) waters, and are used during stratified and non-stratified conditions. Though mixed reservoirs do not technically host epilimnia and hypolimnia, these terms are still used to describe relative depths during the wet season.

7 Special Study 1: Comparative Study

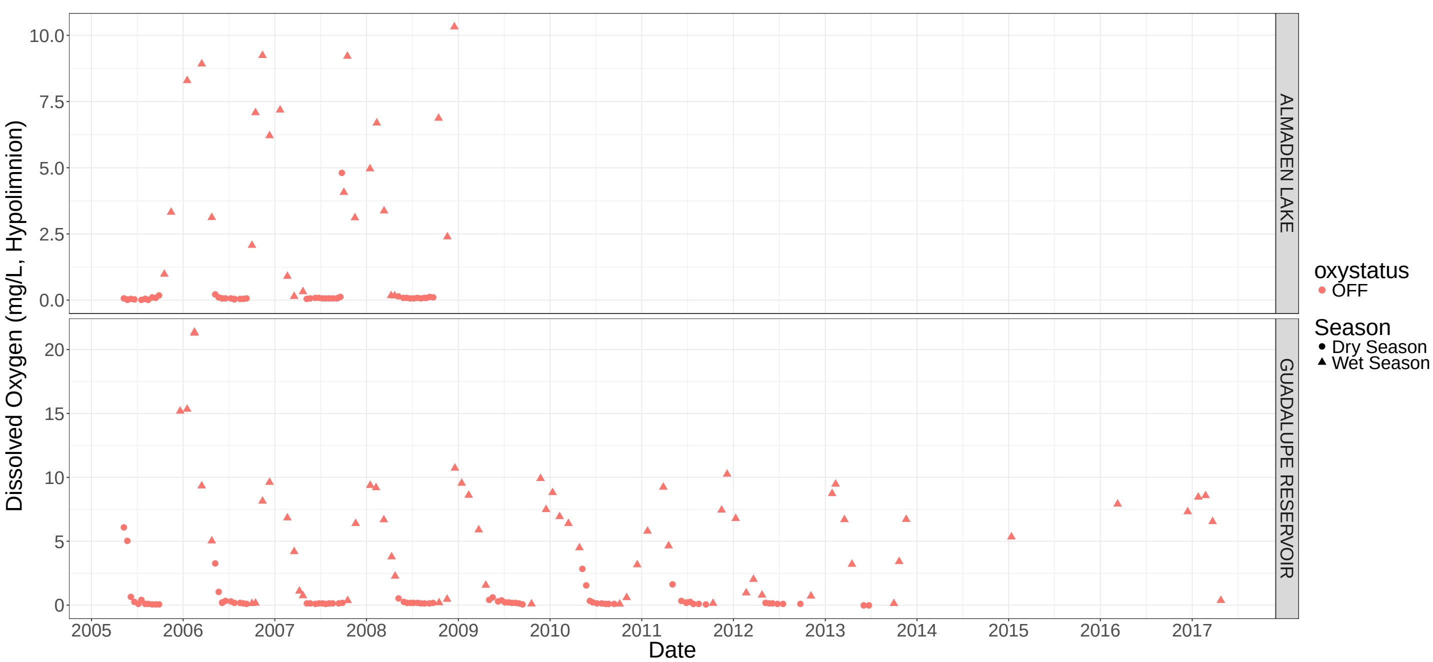
Section 9.10 of the Guadalupe River Watershed TMDL Staff Report presents the following study question:

"How do the reservoirs and lakes in this watershed differ from one another? Factors to consider include, but are not limited to, area of connected wetlands, food web, water chemistry (phosphorus, pH, acid neutralizing capacity, and dissolved organic carbon), water level fluctuations, and infrastructure (outlet structure). Do outlet samples adequately represent hypolimnetic methylmercury concentrations for each reservoir? How significant are these differences?"

In addition to the reservoirs and lakes in the Guadalupe Watershed, this section includes discussion of the Stevens Creek Reservoir reference site. In this section, we report data collected prior to the resetting of the solar circulators in 2009 at Site 1 of Almaden Lake. Reservoir data reported was collected during the wet season when hypolimnetic oxygenation systems are not operated, and during the dry season prior to the operation of the systems (Figure 1).

For parameters that contain multiple non-detect values reflecting variable reporting limits (total phosphorus, nitrate, and ammonia), modeled mean concentrations are reported. These were calculated using the "NADA" package for the R computing language as modeled estimates based on empirical cumulative distribution functions (ECDF) for censored data using the Kaplan-Meier method. For parameters that contained fewer than 5% non-detect values, medians are reported to reflect characteristic values with minimal influence of extreme values.

Figure 1: Example of Data Used in Special Study 1 for Lake And Reservoirs



7.1 Water Source and Storage Capacity

Water source is a major determinant of a reservoir's total mercury concentration. Mercury can be introduced to water bodies through discharge of industrial or mining wastes, weathering of naturally-occurring minerals containing mercury, or from atmospheric deposition. The five water bodies described in this section receive inflow from various sources, incorporating each origin described above.

Guadalupe River Watershed Table 3 describes storage capacities and water sources of the mercury-impaired water bodies and the reference site. Almaden, Calero, and Guadalupe reservoirs were constructed in the 1930s

Table 3: Water Source and Storage Capacity

Reservoir	Capacity (acre-feet)	Source	Catchment Area (acres)	Mean Depth (ft)	Max. Depth (ft)
Almaden Reservoir	1,586	Local	7,666	32	66
Calero Reservoir	9,934	Imported and Local	4,676	34	66
Guadalupe Reservoir	3,415	Local	3,812	60	76
Almaden Lake	586	Local	24,465	18	40
Stevens Creek Reservoir	3,138	Local	11,043	36	79

to increase Santa Clara County's water storage. Almaden and Guadalupe reservoirs receive water from natural inflow sources, while Calero Reservoir primarily receives imported water from the Sacramento-San Joaquin River Delta, transferred from San Luis Reservoir through the Santa Clara Conduit. San Luis Reservoir stores water shared by the State Water Project and federal Central Valley Project, and is listed as impaired for mercury [2]. The Almaden-Calero canal delivers water from Almaden Reservoir to Calero Reservoir. Guadalupe and Almaden reservoirs are located adjacent to the New Almaden Mining District, and have received mercury-laden sediment and waste material directly, while Calero Reservoir is contaminated by water transported through the Almaden-Calero canal and Santa Clara Conduit, as well as through atmospheric deposition (Appendix A.2).

Almaden Lake is a 32-acre impoundment along Los Alamitos Creek, created in the 1950s and 1960s due to in- and off-stream quarrying. As a result, the lake has an irregular bathymetry with few relatively deep pits separated by shallower sections (Appendix A.7). Los Alamitos Creek receives inflow from Almaden Reservoir, Calero Reservoir, and urban runoff from residential parcels.

Reference Site Stevens Creek Reservoir was one of the six original reservoirs approved for construction by Santa Clara County voters in 1934. With a capacity of 3,138 acre-feet, it is similar in size to Guadalupe Reservoir, though shallower and broader. Stevens Creek Reservoir is located outside of the Guadalupe Watershed and does not receive inflow from creeks effected by mercury mining. The reservoir is fed by Montebello Creek, Swiss Creek, and Stevens Creek, as well as various small drainages.

7.2 Infrastructure and Engineering

Dam construction is known to accelerate the production and bioaccumulation of methylmercury, resulting from microbial decomposition of terrestrial organic matter and subsequent hypolimnetic anoxia [14]. The depth of water withdrawal can alter a reservoir's stratification regime, which can influence methylmercury production [22]. Hypolimnetic withdrawal may cause a net-warming effect, increasing the size of the epilimnion, and decreasing a reservoir's thermal stability. This could result in vertical entrainment of profundal compounds into the upper water column [35].

Guadalupe River Watershed Almaden Reservoir impounds water in the valley produced by Alamitos Creek and Jacques Gulch. Its earthen dam stands 110 feet tall with a crest of approximately 500 feet in width, and an elevation of 615 feet above sea level. Guadalupe Dam is a 129-foot-high, 650-foot-wide earthen embankment that impounds water in the valley produced by Guadalupe Creek. Its crest reaches an elevation of 625 feet. Calero Reservoir's two dams impound water along Calero and Cherry Canyon creeks. Its primary 90-foot-high earthen dam spans 840 feet wide. Calero Reservoir's secondary dam is located at the northern end of the reservoir, and impounds water behind a 500-foot-wide, 40-foot-tall earthen embankment. Both of Calero Reservoir's dams share a crest elevation of 490 feet.

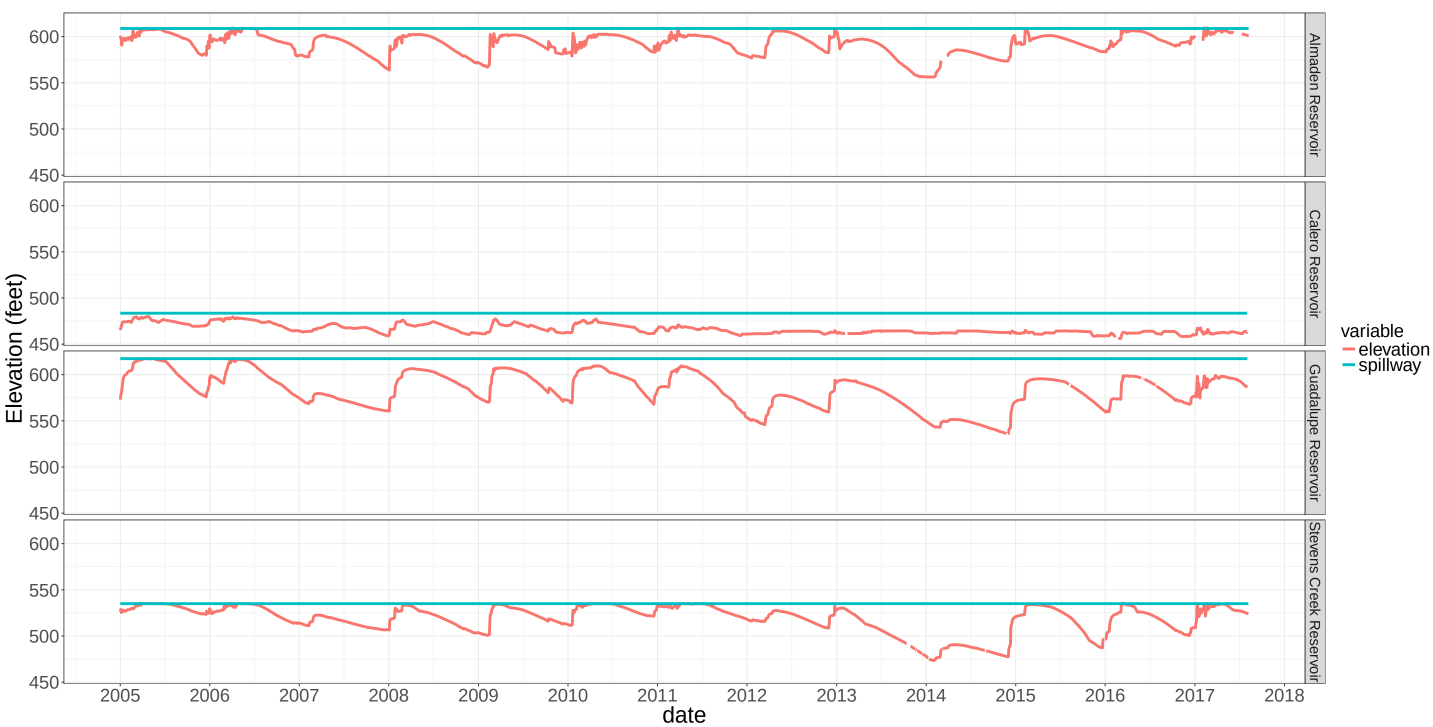
Reservoir outlet works are of the bottom-release penstock variety, with 36" concrete-encased steel pipes extending under the dams and discharging water downstream. Almaden Lake's water level is maintained by the discharge of Alamitos Creek, which varies seasonally and is influenced by outflow from Calero and Almaden reservoirs. A wood-paneled flashboard dam is deployed periodically to divert water into the Alamitos Percolation Ponds (Appendix A.9). A proposed capital project that intends to separate Almaden Lake from Alamitos Creek is currently under environmental and engineering review. This project plans to reduce methylmercury production and eliminate the lake's thermal barrier to steelhead trout passage.

Reference Site Stevens Creek Reservoir impounds water in the narrow valley of Stevens Creek. Constructed during the same period as the reservoirs of the Guadalupe River watershed, it shares a similar variety of penstock outlet works with a 36" concrete-encased steel pipe. Its earthen dam was raised 10 feet in 1985, bringing it to a height of 120 feet above the valley floor. The dam spans 480 feet, making it morphologically similar to Almaden Dam. Slightly lower in elevation than Almaden and Guadalupe dams, Stevens Creek Dam's crest sits 555 feet above sea level.

7.3 Water Level Fluctuation

The State Water Resources Control Board has found a sigificant correlation between water level fluctuation and elevated mercury concentrations in fish [3]. Water level fluctuation has been suggested to enhance methylation in seasonally-inundated reservoir margins by oxidatively increasing sulfate concentrations while sediments are exposed to the atmosphere. These increased sulfate concentrations may stimulate methylation by sulfate-reducing bacteria when submerged [33]. Water level fluctuation can also increase partitioning of inorganic mercury from the solid to porewater phase, making it more bioavailable, and increase dissolved organic carbon in the porewater, stimulating microbial activity [34]. In oligotrophic lakes, benthic primary productivity can be a factor limiting fish growth [50]. Frequent water level fluctuations may erode nutrient-rich substrates that support benthic algal production, potentially restricting fish growth dilution through cultural oligotrophication. Concurrently, fish may become more dependent on the pelagic food web, which is known to host greater bioaccumulation [72].

Figure 2: Water Level Fluctuations of Reservoirs



Results: Guadalupe River Watershed Figure 2 shows historical fluctuations in surface elevation occurring in the reservoirs. Almaden, Calero, and Guadalupe reservoirs experience varying degrees of water level fluctuation due to their inflow levels and provenance, geometries, storage capacities and restrictions, and regulated outlet flows. Average annual capacity fluctuation is defined as the arithmetic mean of water-yearly capacity variations, measured as the difference between maximum and minimum annual capacity as a percentage of total reservoir capacity. The largest of the three reservoirs, Calero Reservoir experiences the lowest variation in water level, with an annual average fluctuation of about 21% of the reservoir's capacity from 2010 to 2016. Calero Reservoir's storage is largely dependent on water imported from San Luis Reservoir, causing low seasonal variation in capacity. Almaden Reservoir's average annual fluctuation was about 67%, as compared with Guadalupe Reservoir's 47%, owing to its smaller storage capacity. In 2010, the California Division of Safety of Dams (DSOD) imposed capacity restriction on Almaden, Calero, and Guadalupe Reservoirs due to seismic concerns. Since this mandate, reservoir storages have been maintained at less than or equal to 80%, 58%, and 80%, respectively, with brief exceedances due to winter storms. Concurrently, average annual capacity fluctuations have decreased in Calero Reservoir by 50% and Guadalupe Reservoir by 20%, but not in Almaden Reservoir. This is likely a combined result of the capacity restrictions and decreased storage due to the 2011 to 2016 California Drought.

Almaden Lake's water level is influenced mainly by the discharge level of Los Alamitos Creek and the erection of the Alamitos Flashboard Dam, which diverts water from the Guadalupe River into the Alamitos Percolation Ponds. When the flashboard dam is deployed, Almaden Lake's water level can increase up to four feet. At these times, the floating solar circulators do not penetrate as deeply into the water column, potentially reducing effectiveness. The relatively modest volume of Almaden Lake and the lack of a permanent barrier for impounding water result in much smaller water level fluctuations than those that occur in the reservoirs.

Results: Reference Site Stevens Creek Reservoir experienced an average annual capacity fluctuation of 54% (Figure 2). There are no existing capacity restrictions imposed on Stevens Creek Reservoir.

Figure 3: Barren Marginal Sediments Resulting from Seasonal Water Level Fluctuation in Guadalupe Reservoir

Discussion In California's semi-arid climate, reservoirs are typically managed to retain large amounts of runoff during the wet season, which slowly deplete throughout the summer until the following winter's rains. This relatively rapid and dramatic water level fluctuation can strip reservoirs of benthic primary productivity, leaving barren sediments around shore margins, as shown in Figure 3. These areas can increase sulfate and dissolved organic carbon concentrations, potentially increasing methylation efficiency of sulfate-reducing bacteria, and decreasing biodilution. Almaden Lake experiences little fluctuation in water level, varying less than a meter annually, mainly due to installation of the Alamitos flashboard dam. Its steep margins minimize dry-back and re-inundation of shoreline sediments. Calero Reservoir, relying predominantly on imported water, receives minimal surface inflow, causing low seasonal variability in storage capacity. Almaden and Guadalupe reservoirs are geographically adjacent, and Almaden Reservoir's catchment area exceeds that of Guadalupe Reservoir by two-fold. Guadalupe Reservoir experiences a lower annual capacity fluctuation than Almaden Reservoir, due to its larger volume and smaller catchment area. Almaden Reservoir's dramatic variation in water level may stimulate methylation in its connected wetlands. Stevens Creek Reservoir experiences similar degrees of water level fluctuation as Guadalupe Reservoir.

7.4 Wetland Connectivity

Wetlands are important locales for mercury methylation and bioaccumulation [36]. Seasonally-flooded wetlands generally experience greater methylmercury production than permanently inundated wetlands [68]. Accelerated decomposition processes in wetlands enrich them with dissolved organic carbon (DOC). Concentrations of total mercury and methylmercury have been documented to increase with DOC and percent near-shore wetlands of a lake's drainage basin [30]. Thus, the wetland connectivity of a reservoir is an important factor that may influence bioaccumulation.

Results: Guadalupe River Watershed The steep topography of the valley that comprises Guadalupe Reservoir causes it to be essentially devoid of wetland habitat. Downstream of its confluence with Los Capitancillos Creek, Guadalupe Creek channelizes into the narrow inlet that feeds the reservoir. Guadalupe Reservoir's frequent water level fluctuations cause this narrow channel to dry and re-inundate seasonally, discouraging the colonization of wetland vegetation. Additionally, the reservoir's steep margins provide little surface for emergent vegetation to take root (Appendix A.10). The United States Fish and Wildlife Service (USFWS) National Wetland Inventory records no wetland habitat in the reservoir.

Almaden Reservoir's relatively broad floodplain near the inlets of Alamitos Creek and Jacques Gulch allow modest wetland formation in the upper reservoir. The National Wetland Inventory suggests the existence of 8.25 acres of emergent and forested/shrub wetlands in this area (Appendix A.12). The District conservatively estimates 3 acres of wetland vegetation, primarily consisting of partially submerged trees and shrubs with minimal herbaceous hydrophytes. This area has not been investigated as a source of methylmercury.

The hilly topography surrounding Calero Reservoir prevents the widespread colonization of wetland vegetation, but small patches of emergent vegetation exist primarily in the upper reaches of the reservoir, and near the Cherry Canyon Creek inlet (Appendix A.13). These areas consist primarily of cattails, and are located up-reservoir of the Almaden-Calero Canal (the primary source of Calero's mercury contamination). The National Wetland Inventory records 5.2 acres of emergent wetland surrounding the reservoir. The District estimates an additional 2.5 acres, though wetland area varies seasonally based on reservoir stage. Calero Reservoir's outlet and spillway drain to a 4.5-acre pond before channelizing into Calero Creek. This area is heavily vegetated with herbaceous hydrophytes.

Excluding a sparse collection of cattails near its inlet, Almaden Lake hosts little wetland vegetation. Due to previous quarry operations, the lake's margins drop steeply away from the shore, creating little space for colonization (Appendix A.7). The reach of the Guadalupe River downstream of the confluence of Alamitos and Guadalupe creeks is heavily vegetated with cattails. When the flashboard dam is deployed, it becomes inundated, and backs up into Almaden Lake (Appendix A.9). At these times, this wetland area may contribute methylmercury to the lake or downstream waters.

Results: Reference Site Stevens Creek Reservoir's dramatic water level fluctuation and steep topography along its margins prevent the colonization of emergent vegetation (Appendix A.11). Near the main reservoir inlet, high water levels tend to partially submerge terrestrial vegetation and trees, but the area is devoid of typical wetland vegetation. The USFWS National Wetland Inventory records no wetlands in or adjacent to the reservoir.

Discussion Almaden Reservoir hosts a higher percentage of connected wetlands (relative to its surface area) than the other water bodies. Located near its inlet, these wetlands are far from the diffuser line of the hypolimnetic oxygenation system, and are likely unaffected by system operation. This area may be a zone for methylation, contributing to the reservoir's high fish tissue mercury concentrations.

7.5 Limnology and Stratification

Thermal stratification occurs predominantly during the dry season when solar radiation warms the surfaces of lakes and reservoirs. Since water is a poor thermal conductor and increases in density with decreases in temperature, water bodies separate into distinctive density layers. The warm, buoyant epilimnion floats atop the cold, dense hypolimnion, separated by a layer of steep temperature gradient known as the metalimnion. In productive systems with limited light penetration, the hypolimnion becomes isolated from the atmosphere and photosynthetic organisms that produce oxygen. Microbial decomposition of organic matter depletes oxygen concentrations in the hypolimnion, producing ideal conditions for the proliferation of anaerobic bacteria responsible for methylmercury production [25]. Stability and duration of thermal stratification, dictating length and severity of hypolimnetic anoxia, are important factors controlling methylmercury production in reservoirs. When the air begins to cool, the temperature of the epilimnion decreases, increasing its density. The metalimnion begins to descend, entraining hypolimnetic waters upward. Once the density of the epilimnion reaches that of the hypolimnion, the remaining bottom waters are forced upward, mixing the reservoir. This phenomenon, termed "turnover," is thought to introduce profundal compounds into surface waters.

Results: Guadalupe River Watershed Almaden Reservoir has historically experienced the shortest seasonal duration of stratification, with periods of hypolimnetic hypoxia (DO $<$ 3 mg/L) generally ranging from two to four months (Figure 4). As the reservoir with the least storage, Almaden is the most susceptible to turbulence due to inflow and natural advection. The hilly topography surrounding the reservoir blocks it from sunlight for a portion of the day.

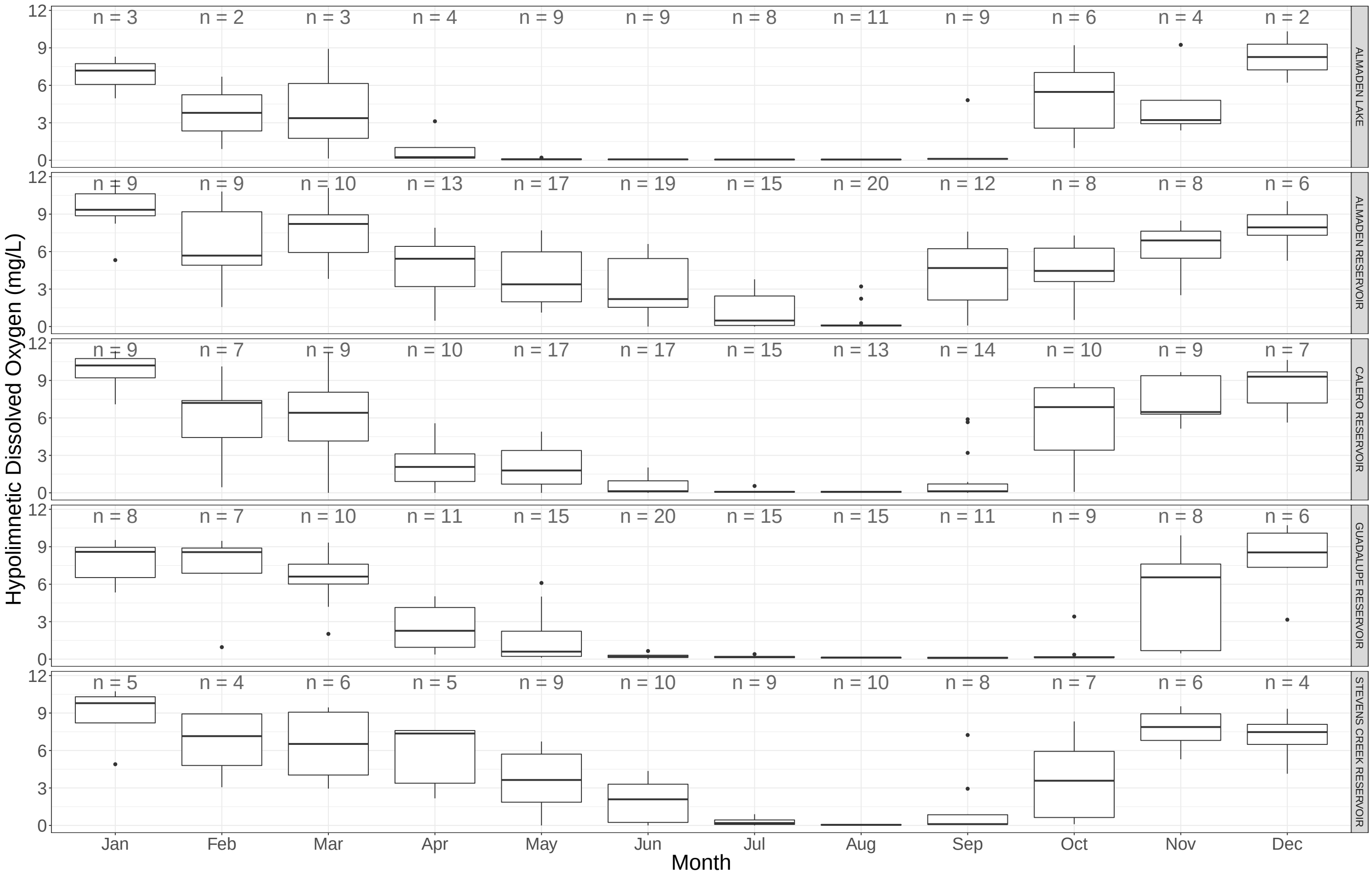
Calero Reservoir experiences a longer period of stratification than Almaden Reservoir, ranging from four to six months (Figure 4). With storage capacity over five-times that of Almaden Reservoir and a mainly imported water supply, Calero Reservoir is less susceptible to circulation and oxygenation due to inflow and natural advection. It has a relatively high surface area to depth, and receives direct sunlight for much of the day. Due to its lowland location, wind speed is often relatively high at Calero Reservoir, potentially enhancing mixing and shortening the period of stratification.

Guadalupe Reservoir experiences the longest period of seasonal thermal stratification, averaging from six to eight months (Figure 4). Guadalupe Reservoir has a low surface area relative to its depth, resulting in high stability and a prolonged period of stratification. The steep topography and north-to-south orientation of the valley in which Guadalupe Reservoir is located minimizes wind-induced mixing and sun exposure. Guadalupe Reservoir's outlet intake is located approximately 3 meters above the reservoir bottom, resulting in a stagnant hypolimnetic pool that often persists for several months. Relatively undisturbed by reservoir outflow, this pool remains isolated from the rest of the water column and likely contributes to an anomalously long period of stratification.

Almaden Lake predominantly impounds water in two abnormally deep pools that served as gravel extraction pits during the quarrying operations of the 1950s and 1960s (Appendix A3). These sites have historically

Figure 4: Hypolimnetic Dissolved Oxygen Concentrations of Reservoirs and Lake

Boxplots include several years of data collected prior to the resetting of the circulator at Site 1 of Almaden Lake, and during periods of nonoperation of the oxygenation systems in the reservoirs.



remained stratified longer than the additional three sampling sites, which are shallower and closer to the main channel of Alamitos Creek. Hypolimnetic dissolved oxygen concentrations in Almaden Lake vary in response to air temperature, Alamitos Creek discharge, and flashboard dam activity. Typically, the hypolimnia of sites one and two remain hypoxic for six to seven months (Figure 4).

Results: Reference Site Stevens Creek Reservoir typically remains thermally stratified for a period of four to six months (Figure 4). Though similar in capacity, its relatively high surface area to depth may cause its thermal structure to be less stable than Guadalupe Reservoir's. The north-to-south orientation of the valley surrounding the reservoir limits sun exposure, potentially shortening the duration of stratification.

Discussion All reservoirs described in this study are monomictic, experiencing varying periods of thermal stratification during the dry season, and mixed conditions following fall turnover. The reservoirs begin to stratify during in early spring, and their thermoclines slowly descend throughout the dry season until mixing occurs. Hypolimnetic oxygen depletion occurs in each water body during periods of stratification. Deeper, narrower reservoirs such as Guadalupe and Stevens Creek reservoirs exhibit strong thermoclines and corresponding oxyclines, while shallower water bodies such as Almaden Lake and Calero Reservoir exhibit weak thermoclines, but strong oxyclines (Figure 5). Strength of stratification appears to correlate with the reservoir's Osgood Index (the ratio of mean depth(m) to the square root of surface area (km^2) (Figure 4). The Osgood index infers the strength of a lake's resistance to mixing, with higher values indicating strongly-stratified reservoirs with little probability for periods of hypolimnetic entrainment [61]. Though all of the water bodies described in this study would be considered shallow and unstable, variations in morphology likely control their duration and strength of stratification.

Almaden Reservoir experiences the shortest period of hypolimnetic anoxia, likely due to its small volume and upland location. Calero and Stevens Creek reservoirs experience similar durations of stratification, ranging from four to six months. Guadalupe Reservoir and Almaden Lake experience longer durations of hypolimnetic anoxia, ranging from six to eight months. Guadalupe Reservoir's prolonged season of stratification is likely a result of its unique outlet structure and high thermal stability. Almaden Lake's long duration of hypolimnetic anoxia can be attributed to its persistent exposure to solar radiation due to its lowland topography, and abundant algal biomass that depletes oxygen concentrations when decomposed.

Figure 5: Typical August Temperature and Dissolved Oxygen Profiles of Reservoirs and Lake

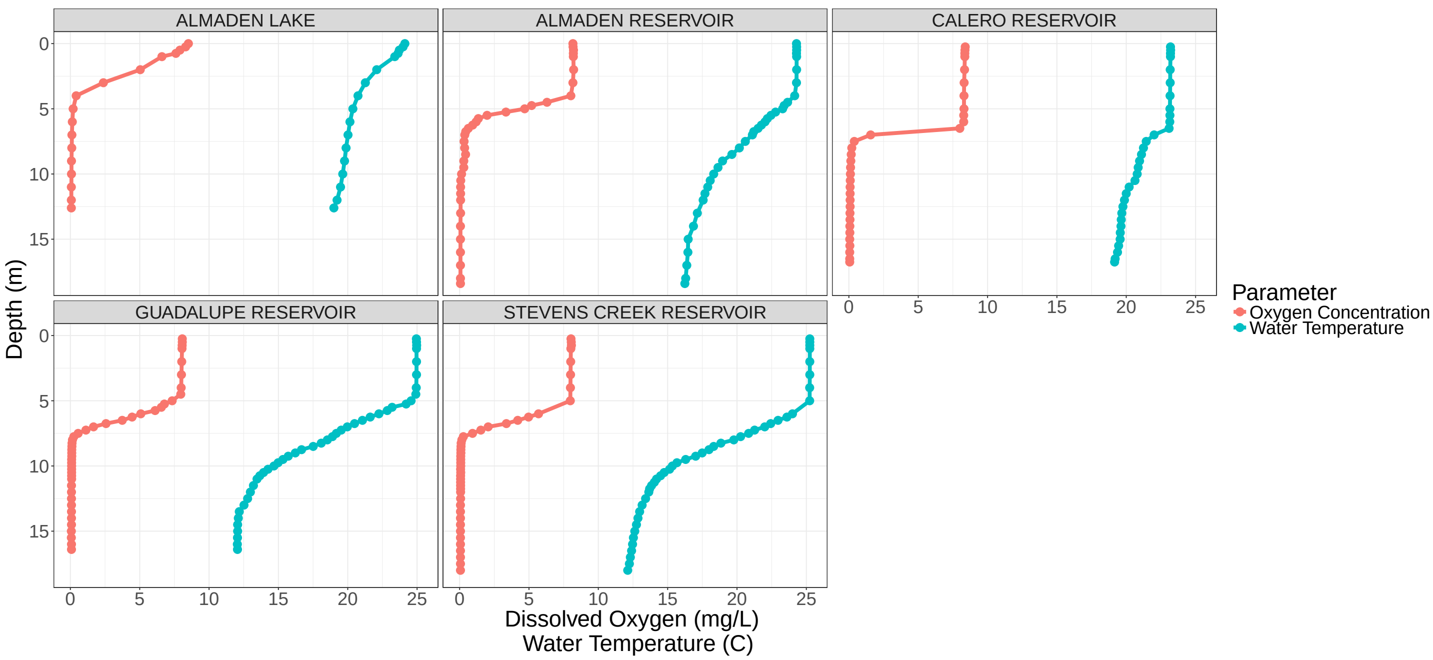


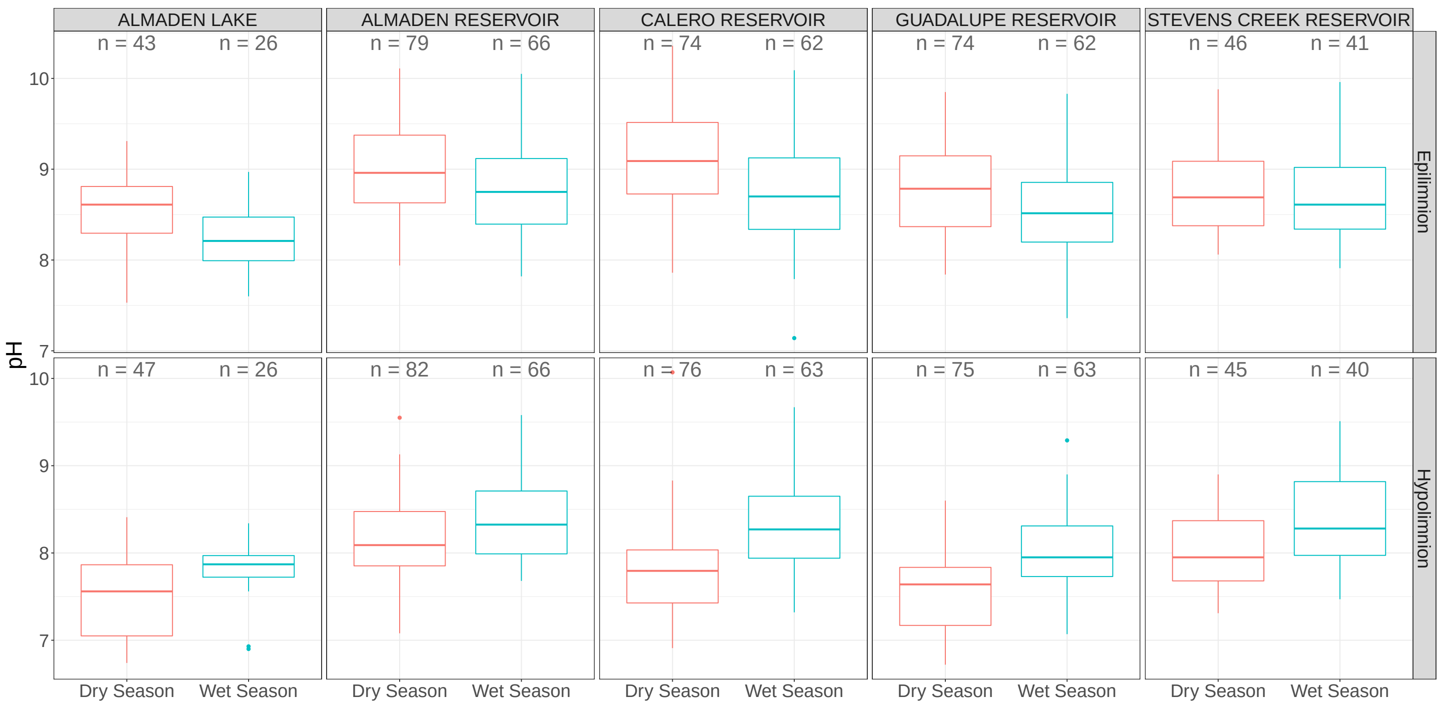
Table 4: Osgood Index of Reservoirs and Lake

Reservoir	Osgood Index
Almaden Reservoir	0.022
Calero Reservoir	0.009
Guadalupe Reservoir	0.038
Almaden Lake	0.015
Stevens Creek Reservoir	0.019

7.6 Alkalinity and pH

The effects of pH on methylmercury production and bioaccumulation are disputed. Acidic conditions can increase the solubility of Hg(0), making it more available for oxidation to Hg(II), and ultimately for methylation by sulfate-reducing bacteria [19]. Low pH can also increase the solubility of methylmercury, making it more available for uptake into biota. Acid mine drainage, common among sulfide-bearing mines, can accelerate dissolution of Hg(0), increasing its bioavailability. Observational studies of acidic lakes in the Adirondack Region region have documented increased aqueous mercury, methylmercury, and fish tissue mercury concentrations with decreasing pH [31]. Some propose that methylation of Hg(II) is pH-dependent, suggesting that more production could occur under acidic conditions [29] [74]. However, other studies document diminished methylation with increasing acidity, likely due to decreased inorganic mercury in porewater, presumably due to the formation of insoluble HgS. This suggests that enhanced methylation is not responsible for increased mercury levels in fish from acidic lakes [63]. In this case, fish tissue mercury concentrations may be higher in acidified lakes due to decreased primary productivity and fish growth rate, diminishing biodilution [29]. Furthermore, increasing pH can result in increased solubility of organic material surrounding lakes, providing dissolved organic carbon known to enhance methylation [46].

Figure 6: Epilimnetic and Hypolimnetic pH of Reservoirs and Lake



Results: Guadalupe River Watershed Mesozoic-aged Franciscan Formation dominates the geology of the New Almaden Mining District (Appendix A.14). This geologic formation consists largely of limestone and carbonate strata. The mineral cinnabar is associated with the metamorphosed limestone and carbonate rocks. The soils of the region are characteristically alkaline, contributing to the slightly-basic pH values ubiquitously measured in the reservoirs and Almaden Lake (Figure 6). Hypolimnetic pH is lower during the dry season ($p<0.001$), a result of increased CO_2 loading due to bacterial degradation of organic matter, respiration, and methane fermentation [80]. Epilimnetic pH is higher during the dry season ($p<0.001$), due to enhanced algal CO_2 utilization for photosynthesis.

Results: Reference Site The geology of the area surrounding Stevens Creek Reservoir is like that of the upper Guadalupe River Watershed, consisting primarily of Neogene to Quaternary-aged Santa Clara formation, and Franciscan Formation (Appendix A.15). As a result, Steven Creek Reservoir exhibits a slightly-alkaline pH similar to the water bodies of the upper Guadalupe River Watershed (Figure 6).

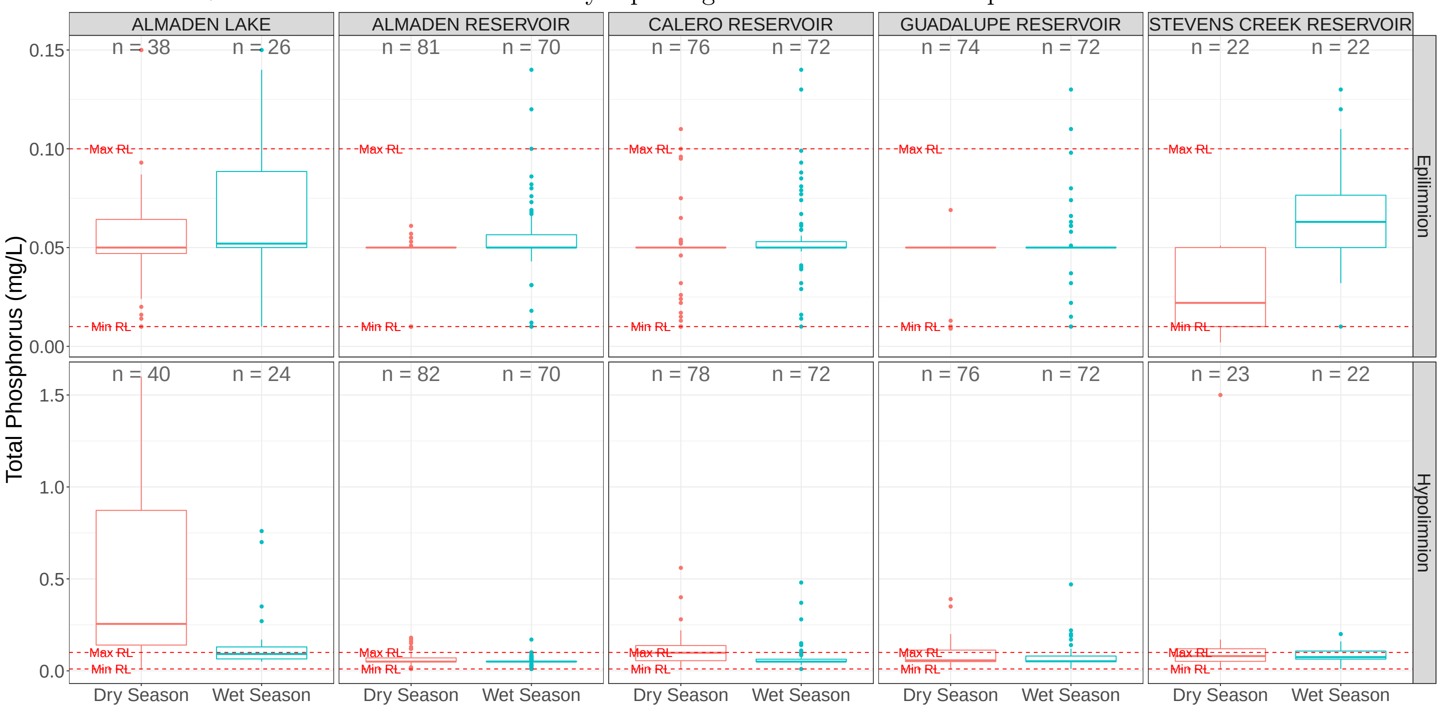
Discussion Because pH varies seasonally due to bacterial degradation of organic matter and photosynthetic utilization of carbon dioxide, wet season epilimnetic pH is the best indicator of background alkalinity of a water body. Though all water bodies of the Guadalupe River Watershed are alkaline, wet season epilimnetic pH is significantly lower in Almaden Lake than in the reservoirs ($p<0.001$). This could be characteristic of the urban runoff that feeds Los Alamitos Creek, or due to the lake's higher primary productivity that occurs during the wet and dry seasons. The absence of the acid mine drainage found in other California mines rich in sulfide minerals decreases the potential dissolution and mobilization of additional mercury present in bedrock and mining waste. Because pH is fairly similar among the water bodies, it is unlikely to drive their differences in mercury cycling.

7.7 Total Phosphorus

Phosphorus is a common limiting nutrient for the growth of algae and aquatic organisms, since it is often the nutrient in shortest supply relative to plant demands. Inorganic phosphorus enters waterbodies and is absorbed by plants and algae, where it is converted to organic forms. Plant and algal biomass and animal excretion containing organic phosphorus settle to lake-bottoms. During decomposition of detritus, organic phosphate is microbially mineralized back to inorganic phosphorus, which forms complexes with iron oxides in profundal sediments. Under anaerobic conditions, bacteria reduce these complexes, releasing ferrous iron and inorganic phosphate into the water column [80]. Thus, external loading of phosphorus is the dominant loading process during the wet season, and internal loading is most dominant during the dry season. Internal loading of phosphorus along with higher water temperatures in the dry season can cause eutrophication, completing the phosphorus cycle by providing additional biomass to stimulate decomposition and sustain hypolimnetic hypoxia. This can promote low-oxygen concentrations ideal for methylmercury production. However, resulting algal blooms can reduce accumulation of methylmercury in aquatic food webs by causing "bloom dilution", decreasing the concentration of methylmercury per algal cell [62].

Figure 7: Epilimnetic and Hypolimnetic Total Phosphorus Concentrations of Reservoirs and Lake

Red dashed lines reflect laboratory reporting limits. Number of samples is shown in black.



Monitoring Results and Discussion Figure 7 shows epilimnetic and hypolimnetic total phosphorus concentrations of the reservoirs and lake measured during the wet and dry seasons. Epilimnetic total phosphorus concentrations were significantly higher during the wet season in Almaden ($p<0.001$), Calero ($p<0.05$), Guadalupe ($p<0.01$), and Stevens Creek reservoirs ($p<0.001$) than during the dry season. This reflects external phosphorus loading through surface runoff during periods of precipitation.

While epilimnetic total phosphorus concentrations were ostensibly higher in Almaden Lake during the wet season than during the wet season, this difference was not statistically significant. Almaden Lake receives urban runoff throughout the year, contributing to the lower seasonal variation in epilimnetic phosphorus concentrations. The lake's shallow morphology may enhance vertical entrainment of profundal compounds during the dry season, providing the photic zone with a relatively constant nutrient load. Almaden Lake and Guadalupe Reservoir host large populations of California Gulls that move periodically between water bodies in Almaden Valley. Termed the

"Almaden Valley Super Flock", these gulls may contribute significant nutrient loading to lakes and reservoirs. A 1995 study estimated that bird droppings accounted 25 to 34% of annual phosphorus loading to an urban lake [69].

Hypolimnetic total phosphorus concentrations were significantly higher during the dry season in Almaden Reservoir ($p<0.05$), Calero Reservoir ($p<0.001$), and Almaden Lake ($p<0.05$) than during the wet season. This indicates internal phosphorus loading from profundal sediments during periods of hypolimnetic anoxia. Guadalupe and Stevens Creek reservoirs did not exhibit statistically significant seasonal differences in hypolimnetic total phosphorus concentrations, but measured values were ostensibly higher during the dry season.

Total phosphorus concentrations were significantly higher in Almaden Lake than in the reservoirs ($p<0.001$), with average concentrations of 0.5 mg/L (SD=0.43) measured in its hypolimnion, and 0.05 mg/L (SD=0.04) measured in its epilimnion during the dry season. Total phosphorus concentrations of this magnitude categorize the lake as eutrophic to hypereutrophic according to Carlson's Trophic State Index [21]. Average total phosphorus concentrations in Almaden Lake exceed those measured in the reservoirs by up to an order of magnitude. This could be a factor of enhanced external loading due to urban runoff of fertilizers and biota contribution from avian feces, or internal loading attributable to the lake's long duration of hypolimnetic anoxia and abundant organic biomass available for decomposition. Because phosphorus is considered a limiting nutrient in aquatic systems, enhanced phosphorus concentrations in Almaden Lake may explain its higher algal productivity. The reservoirs contain statistically similar, lower total phosphorus concentrations. These reservoirs are categorized as mesotrophic to eutrophic.

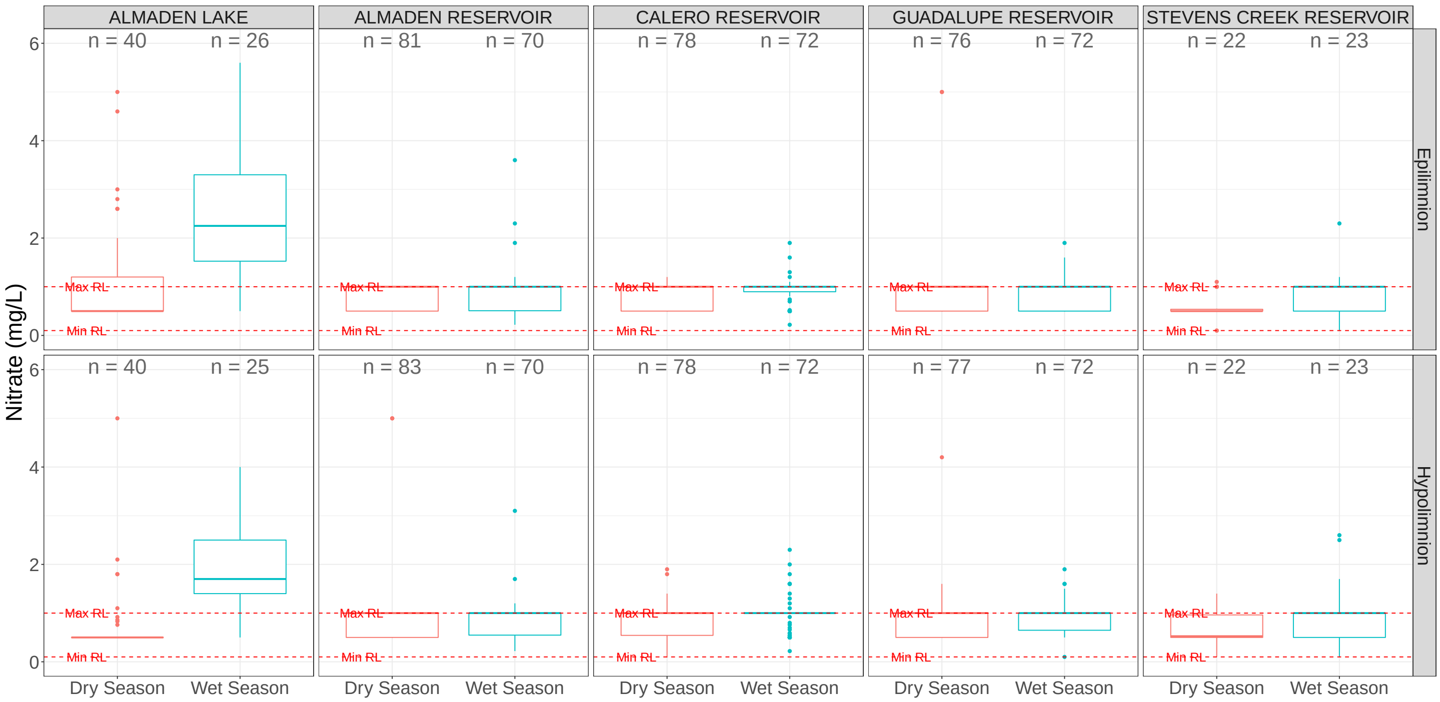
7.8 Nitrate

Nitrate is a common component of synthetic fertilizers. Excessive nitrate concentrations are documented in aquatic ecosystems receiving surface runoff from agricultural or landscaped urban areas. Nitrate is water-soluble, allowing it to move freely throughout surface and groundwater systems. Nitrate forms inside reservoirs through the biological oxidation of ammonia, termed "nitrification". Excessive nitrate in surface water can cause direct toxicity to aquatic organisms [18]. Additionally, nitrate loading can cause biostimulatory effects that promote eutrophication, harmful algal blooms, oxygen depletion, and pH changes. However, nitrate addition in lakes and reservoirs has been shown to reduce phosphorus release from anoxic sediments, enhance oxidation of organic matter, reduce methylmercury production, and reduce hydrogen sulfide accumulation. This is accomplished by altering redox conditions in favor of nitrification, above redox potentials that promote microbial methanogenesis, sulfate reduction, or iron reduction [9]. A 2013 study documented substantially reduced methylmercury concentrations in the water column and aquatic food web of a lake following the addition of neutrally-buoyant liquid calcium nitrate plume to its hypolimnion during thermal stratification [56]. The presence of nitrate in lakes and reservoirs is an important factor determining redox potential, methylmercury production, and bioaccumulation.

Monitoring Results and Discussion Figure 8 shows epilimnetic and hypolimnetic nitrate concentrations of the reservoirs and lake measured during the wet and dry seasons. Epilimnetic nitrate concentrations were significantly higher during the wet season in Almaden ($p<0.001$), Calero ($p<0.001$), and Guadalupe ($p<0.05$) reservoirs, and in Almaden Lake ($p<0.001$). Hypolimnetic nitrate concentrations were significantly higher during the wet season in Almaden Reservoir and Almaden Lake ($p<0.001$). Stevens Creek Reservoir did not exhibit a significant seasonal variation in nitrate concentrations.

Seasonal differences were only of practical significance in Almaden Lake, where average nitrate concentrations more than doubled during the wet season, with an average concentration of 2.6 mg/L in its epilimnion (SD=1.75). The lake's higher nitrate concentrations measured during the wet season suggest external loading through urban runoff containing fertilizers. Oxidation of ammonia under aerobic conditions could also contribute to elevated nitrate concentrations during the wet season. During periods of hypoxia, anaerobic denitrifying bacteria convert nitrate to gaseous nitrogen, and chemotrophic microbes engage in dissimilatory nitrate reduction, potentially decreasing nitrate concentrations in the hypolimnia of lakes and reservoirs [38].

Figure 8: Epilimnetic and Hypolimnetic Nitrate Concentrations of Reservoirs and Lake



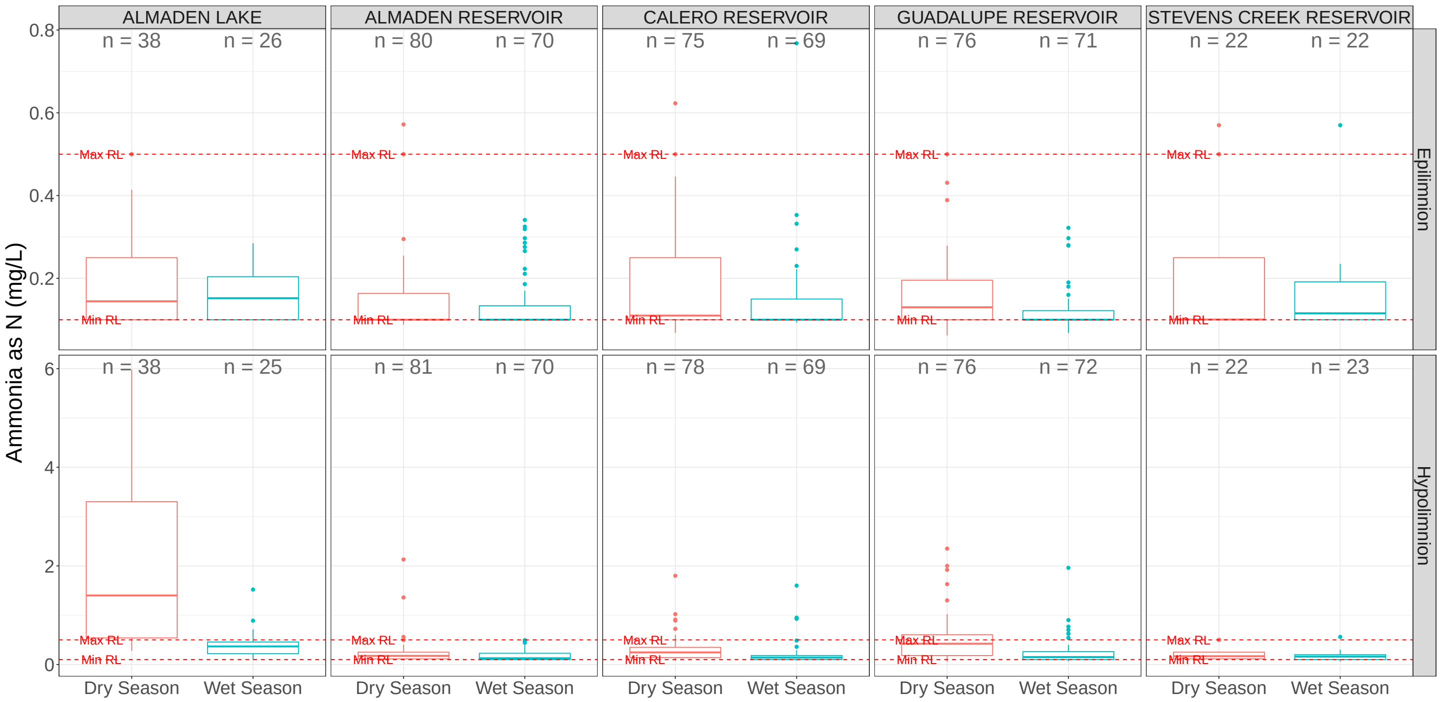
Nitrate concentrations were significantly higher in Almaden Lake than in the reservoirs ($p<0.001$), ranging from 3 to 6-fold greater on average. This likely contributes to the lake's higher algal productivity. Increased algal growth may provide organic detritus available for microbial decomposition, compounding anoxia. Conversely, the abundance of nitrate may poise redox conditions in favor of denitrification, perhaps delaying sulfate reduction following the onset of hypolimnetic anoxia. This could, in turn, delay the onset of methylmercury production in profundal sediments.

7.9 Ammonia

Ammonia is the preferred nitrogenous nutrient for plant and phytoplankton growth. It can be converted to nitrite or nitrate by nitrifying bacteria, making it bioavailable for denitrification, which can remove nitrogen from the aquatic environment through the production of nitrogen gas. Ammonia is toxic to fish and other vertebrates, causing decreased development and reproduction, or death [54]. It can be introduced to aquatic environments through animal excrement or produced by decomposition of organic matter at lake-bottoms. Ammonia is released from anoxic profundal sediments due to decreased nitrification of the compound, and low assimilation rates under anaerobic conditions. Hypolimnetic oxygenation has been shown to reduce accumulation of ammonia in profundal zones of thermally stratified lakes [7].

Monitoring Results and Discussion Figure 9 shows wet and dry season ammonia concentrations measured in the epilimnion and hypolimnion of each water body. Hypolimnetic ammonia concentrations were significantly higher during the dry season in Almaden Lake ($p<0.001$), and in Calero ($p<0.01$) and Guadalupe ($p<0.001$) reservoirs than during the wet season. Almaden and Stevens Creek reservoirs support shorter periods of stratification (Figure 4) and contain less organic biomass available for microbial decomposition, potentially contributing to low seasonal variability in hypolimnetic ammonia concentrations. In all the water bodies, ammonia concentrations were higher in the hypolimnion than in the epilimnion throughout the year ($p<0.001$). This reflects ammonia efflux from profundal sediments.

Figure 9: Epilimnetic and Hypolimnetic Ammonia Concentrations of Reservoirs and Lake



Hypolimnetic ammonia concentrations were highest in Almaden Lake ($p<0.001$), with a mean concentration of 2.3 mg/L (SD=2.3) measured during the dry season. Hypolimnetic ammonia concentrations were considerably lower in Guadalupe Reservoir ($p<0.001$), with a mean concentration of 0.45 mg/L (SD=0.41) measured during the dry season. The other reservoirs contained lower, statistically-similar hypolimnetic ammonia concentrations. Epilimnetic ammonia concentrations were significantly higher in Almaden Lake than in the reservoirs ($p<0.05$), potentially contributing to its enhanced trophic status.

7.10 Sulfate

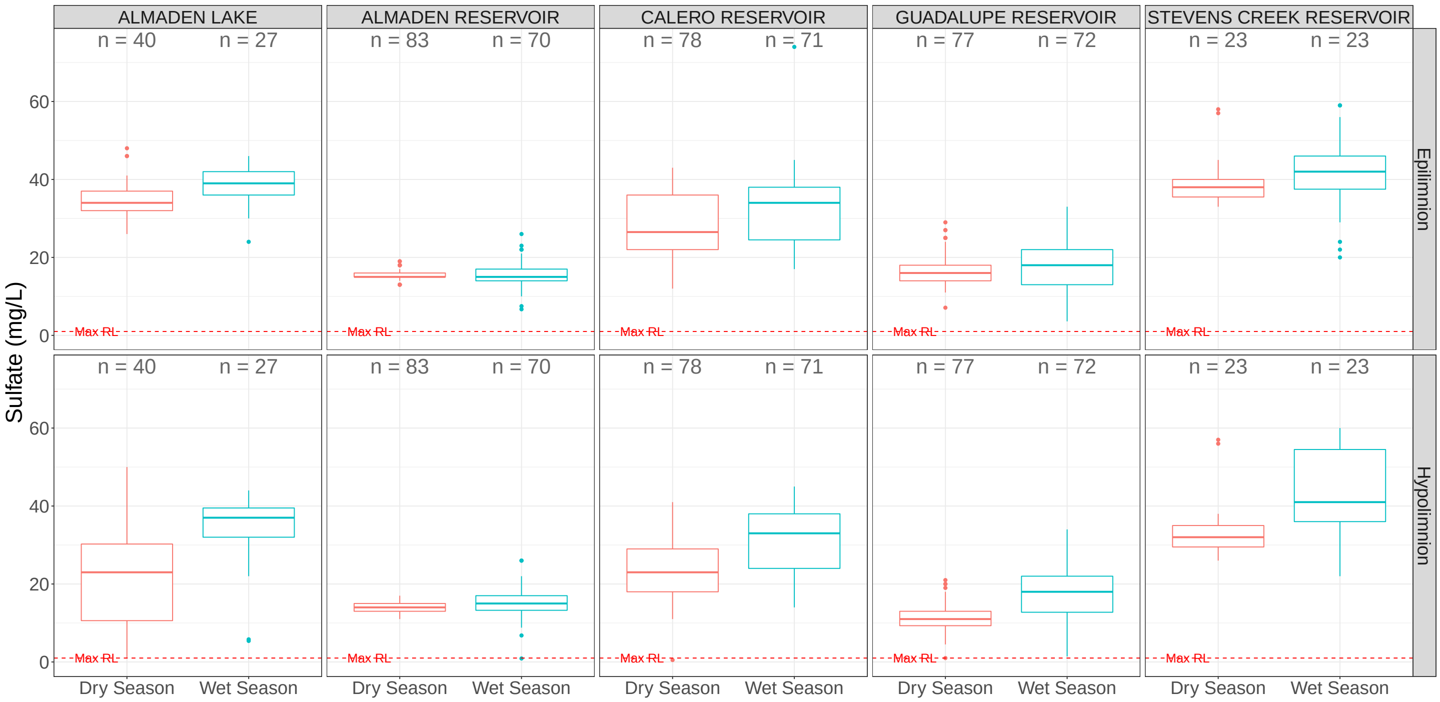
Geologic weathering releases sulfur, which is oxidized to sulfate under aerobic conditions. Sulfur dioxide is released to the atmosphere during volcanic eruptions and from combustion of biomass and fossil fuels. Sulfur can accumulate in lakes and reservoirs due to wet or dry deposition, runoff of sulfur-bearing water or sediments, or acid rain precipitation.

Sulfate is the oxygen source for anaerobic sulfate-reducing bacteria, which are known to be the primary organisms responsible for methylmercury production, along with some iron reducers, methanogens, and Firmicutes containing the *hgcAB* gene cluster necessary for methylation [39]. Sulfate reducers use sulfate as a terminal electron acceptor, producing sulfur or hydrogen sulfide: a toxic, corrosive gas known to cause taste and odor problems in drinking water [59]. Hydrogen sulfide may react with Hg(0) or Hg(II) to form insoluble HgS, reducing bioavailability of mercury for methylation. Therefore, at high sulfate concentrations under reducing conditions, methylation by sulfate-reducing bacteria can be inhibited by the bacteria's own sulfide production [75]. However, formation of charged and uncharged mercury-sulfide complexes can increase bioavailability [47]. Concentrations of 10 to 20 mg/L of sulfate are suggested to facilitate the highest rate of methylmercury production, occurring between sulfate limitation and sulfide inhibition [6].

In addition to stimulating toxic methylmercury and sulfide production, sulfate reduction promotes internal phosphorus loading by mobilizing phosphate from bottom sediments. Hydrogen sulfide can react with iron phosphate, precipitating iron sulfide and mobilizing phosphate [20]. Hypolimnetic oxygenation has been documented to reduce hydrogen sulfide concentrations in reservoirs by curtailing anaerobic sulfate reduction [12]. This could concurrently reduce eutrophication and methylmercury production.

Figure 10: Epilimnetic and Hypolimnetic Sulfate Concentrations of Reservoirs and Lake

Shaded area represents 95% confidence interval for linear regression

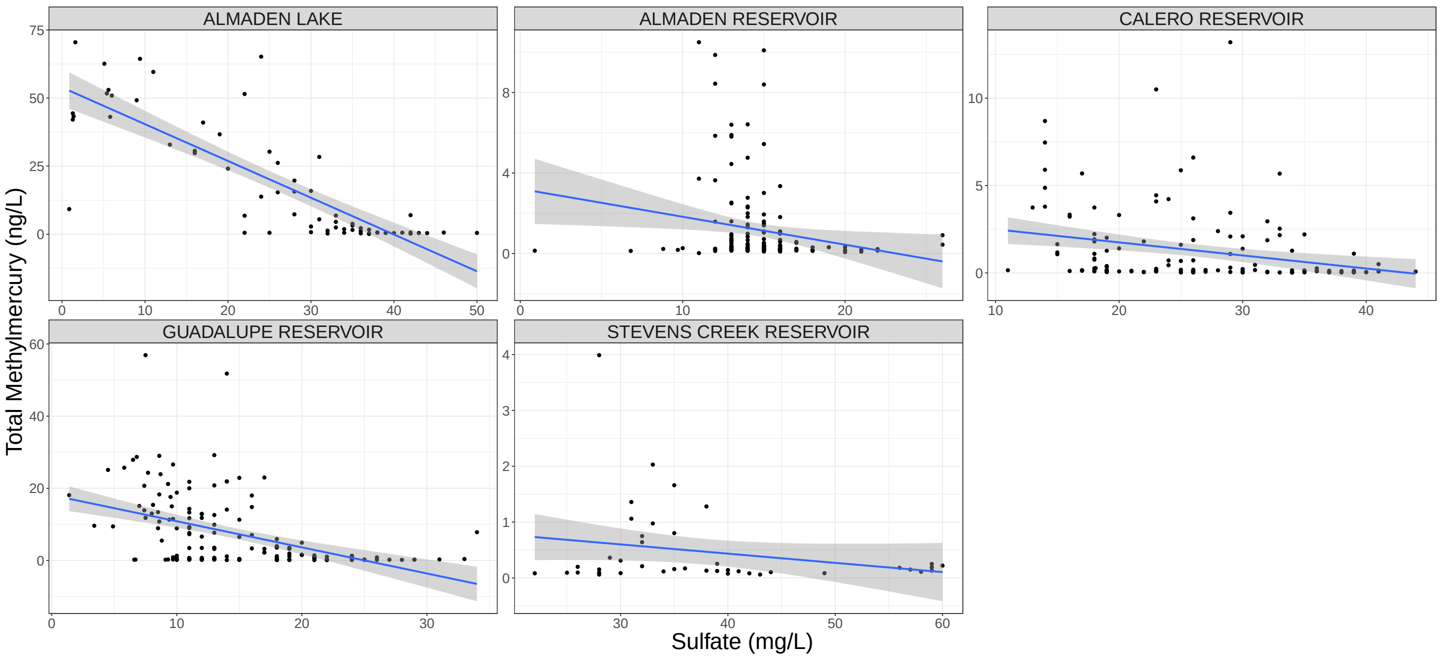


Monitoring Results and Discussion Figure 10 shows wet and dry season sulfate concentrations measured in the epilimnia and hypolimnia of the reservoirs and lake. In each water body, hypolimnetic sulfate concentrations were significantly lower than epilimnetic sulfate concentrations during the dry season, but not during the wet season ($p<0.001$). This vertical sulfate gradient likely reflects the microbial metabolism of sulfate that occurs primarily at the sediment-water interface during periods of hypolimnetic hypoxia. Anaerobic sulfate-reducing bacteria reduce sulfate to hydrogen sulfide, decreasing aqueous sulfate concentrations. During the wet season, reservoirs are mixed, and sulfate concentrations are relatively consistent throughout the water column.

Each reservoir exhibited a significant relationship between hypolimnetic total methylmercury and sulfate concentrations, with methylmercury concentrations decreasing with increased sulfate (Figure 11). Since mercury is converted to methylmercury as a byproduct of bacterial sulfate reduction, sulfate maxima likely reflect decreased microbial activity, and therefore, decreased methylation. That said, sulfate concentration was not a consistently strong predictor of methylmercury concentration in these reservoirs (R^2=0.04 to R^2=0.67). Stevens Creek and Almaden Reservoirs exhibit distinctive sulfate concentration windows that support high methylmercury production (10-17 mg/L in Almaden and 30-40 mg/L in Stevens Creek Reservoir). Sulfate concentrations occurring below these thresholds likely inhibit methylmercury production through sulfide inhibition.

Wet season concentrations are the most appropriate representation of background sulfate levels, since summer sulfate depletion varies with duration and extent of hypolimnetic anoxia. As mentioned previously, there exists no significant vertical sulfate gradient during the wet season, and the compound is distributed homogenously throughout the water column. Almaden and Guadalupe reservoirs had relatively low, statistically-similar wet season hypolimnetic sulfate concentrations, with mean values of 15.2 mg/L (SD=3.8) and 17.6 mg/L (SD=6.9), respectively. Calero Reservoir had significantly higher concentrations ($p<0.001$), with a mean of 30.9 mg/L (SD=8). Almaden Lake had higher sulfate concentrations than Calero Reservoir, but less than Stevens Creek Reservoir. Stevens Creek Reservoir had the highest wet season hypolimnetic sulfate concentrations ($p<0.01$), with a mean of 42 mg/L (SD=12.3) during the wet season. This is likely due to sulfate enrichment in the surrounding geology, or to localized deposition of sulfur dioxide emissions from the nearby Lehigh Southwest Cement Plant.

Figure 11: Hypolimnetic Sulfate and Methylmercury Concentrations of Reservoirs and Lake

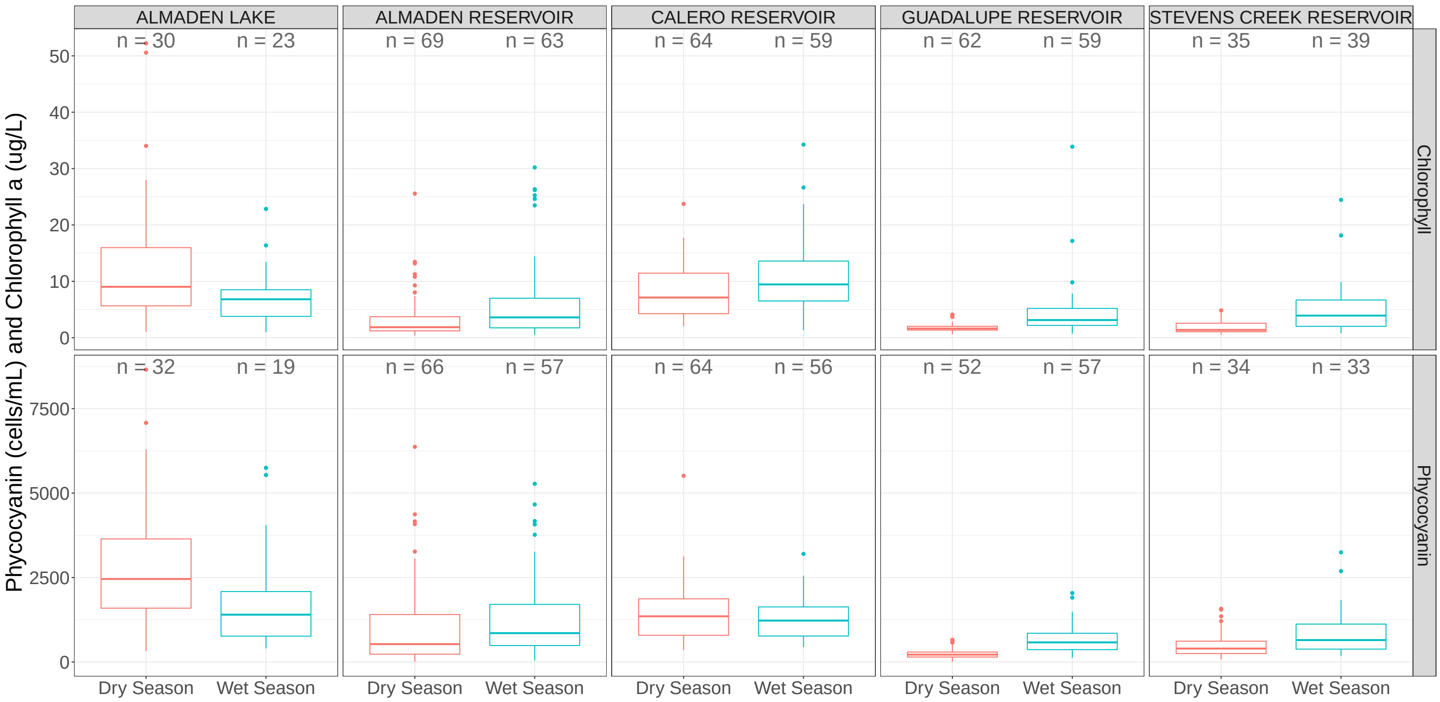


7.11 Chlorophyll *a* and Phycocyanin

Chlorophyll *a* and phycocyanin, are pigments found in phytoplankton and cyanobacteria. Quantifying these pigments present in the water column can suggest the relative abundance of each organism. The largest biomagnification of methylmercury occurs between the water column and phytoplankton, increasing concentrations by a factor of 100,000 [48]. Increased phytoplankton growth has been documented to decrease methylmercury concentrations in zooplankton 2 to 3-fold in a process known as "bloom dilution" [62]. As algal biomass increases, methylmercury concentration per phytoplankton cell decreases, resulting in reduced bioaccumulation in eutrophic lakes and reservoirs. Conditions supporting the development of cyanobacteria are associated with enhanced methylation rates [81]. However, certain species of cyanobacteria are documented to facilitate the conversion of Hg(II) to metacinnabar, reducing its bioavailability [53]. Methylmercury can bioaccumulate in cyanobacteria, potentially diluting concentrations in phytoplankton, but it is toxic to cyanobacteria in high concentrations [23]. Cyanobacteria are not a preferred food source to zooplankton, so accumulation of methylmercury in cyanobacteria may reduce its movement up the food web. A low proportion of chlorophyll *a* to phycocyanin could cause a deficit in quality food available for organisms, restricting growth rates and limiting somatic growth dilution [3].

Monitoring Results and Discussion Figure 12 shows epilimnetic chlorophyll *a* and phycocyanin concentrations measured during the wet and dry seasons. Epilimnetic chlorophyll *a* concentrations were significantly higher during the wet season in Almaden ($p<0.01$), Calero ($p<0.05$), Guadalupe ($p<0.001$), and Stevens Creek ($p<0.001$) reservoirs, and during the dry season ($p<0.05$) in Almaden Lake. Similarly, phycocyanin concentrations were significantly higher during the wet season in Almaden ($p<0.05$), Guadalupe ($p<0.05$), and Stevens Creek ($p<0.01$) reservoirs, and during the dry season in Almaden Lake ($p<0.05$). Wet season primary productivity in the reservoirs likely reflects the effects of fall destratification and winter nutrient runoff, which provide nutrients to the photic zone and support algae blooms. Almaden Lake's shallow morphology makes it susceptible to vertical entrainment of profundal nutrients into the photic zone during periods of anoxia, while its urban drainage area provides nutrient-enriched runoff throughout the year. As a result, turnover and winter runoff do not appear to limit the lake's productivity, and most blooms occur during the summer when abundant solar radiation is

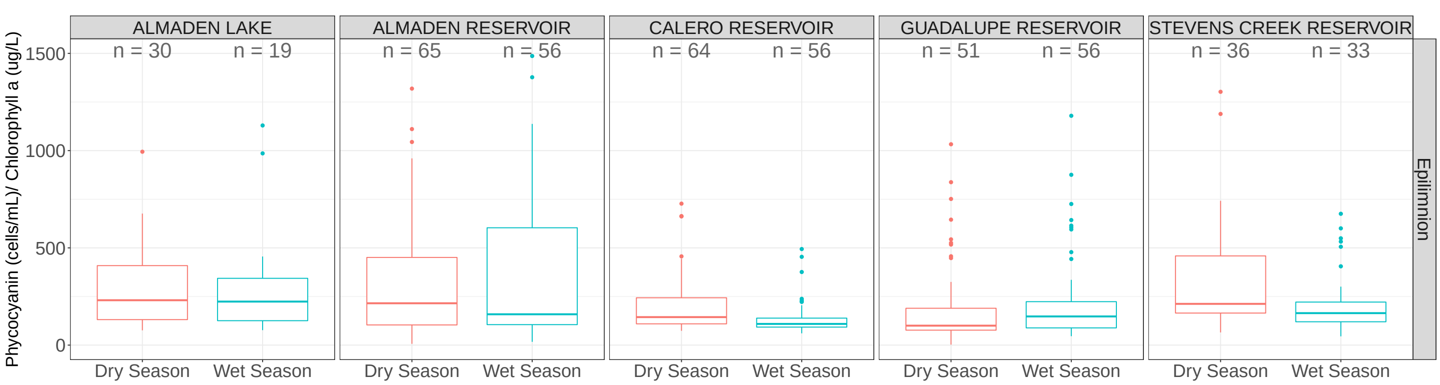
Figure 12: Epilimnetic Chlorophyll *a* and Phycocyanin Concentrations of Reservoirs and Lake



available to photosynthetic organisms.

Almaden Lake and Calero Reservoir contain statistically similar chlorophyll *a* concentrations that are higher than the other reservoirs. This is likely due to their elevated nutrient concentrations, shallow morphologies, and sustained sun exposure. In Almaden Lake, dry season chlorophyll *a* concentrations averaged 13.7 $\mu g\,L^{-1}$ (SD=13), and in Calero Reservoir, wet season chlorophyll *a* concentrations averaged 10.7 $\mu g\,L^{-1}$ (SD=6). These reservoirs would be considered mesotrophic using Carlson's Trophic State Index criterion for chlorophyll [21]. Each reservoir had significantly different phycocyanin concentrations. Almaden Lake contained the highest concentrations, with an average of 2,843 cells/mL (SD=1,950) measured during the dry season, and Guadalupe Reservoir contained the lowest concentrations, with an average of 260 cells/mL (SD=174) measured during the wet season.

Figure 13: Ratio of Epilimnetic Chlorophyll *a* to Phycocyanin Concentrations of Reservoirs and Lake



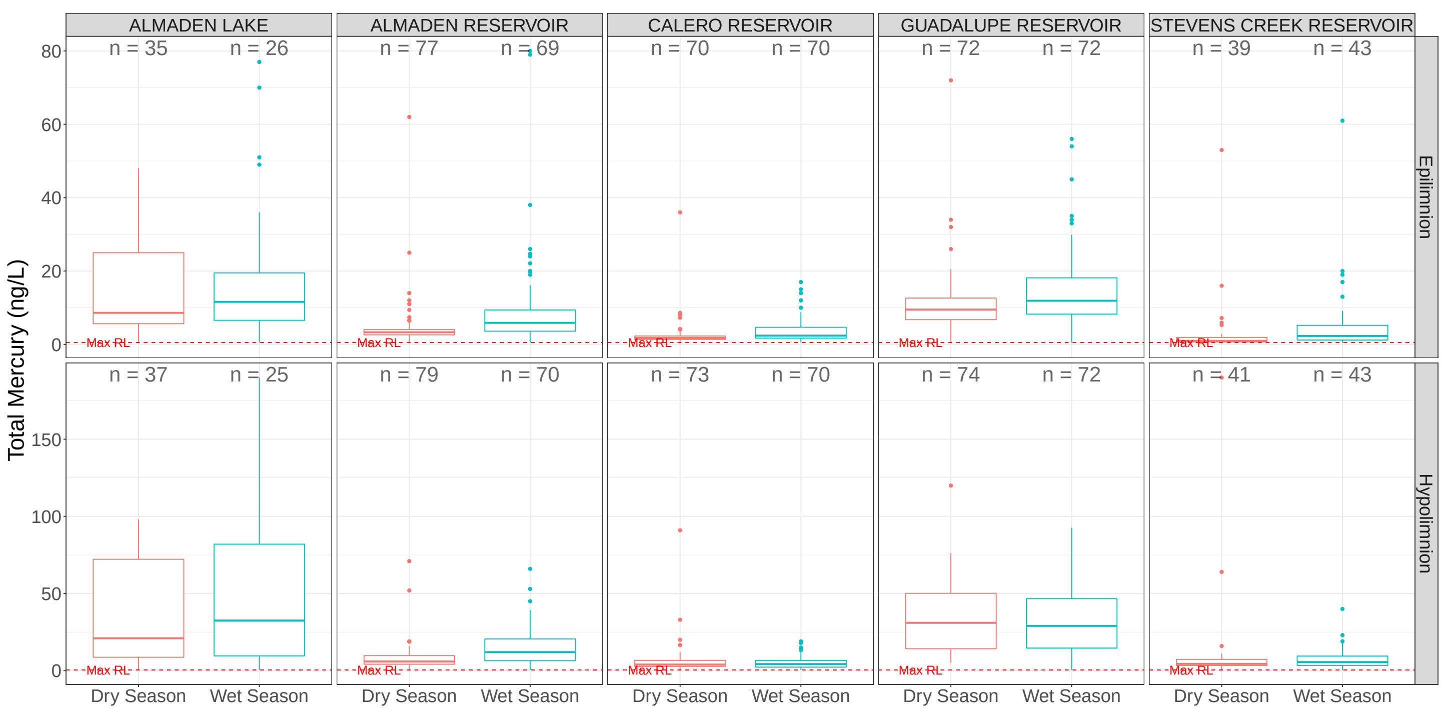
The ratio of chlorophyll *a* to phycocyanin can be used to infer the abundance of quality algal biomass available for uptake by zooplanton. Reservoirs with high relative abundances of cyanobacteria may experience diminished bioaccumulation since less methylmercury would be concentrated in the food source used by zooplankton. Calero and Guadalupe reservoirs contain lower phycocyanin concentrations relative to their concentrations of chlorophyll *a* than do the other reservoirs (Figure 13). This may support bloom dilution by reducing methylmercury concentrations in phytoplankton. Low relative proportions of chlorophyll *a* to phycocyanin may also restrict growth rates of organisms, potentially increasing methylmercury concentrations in fish.

7.12 Total Mercury

Mercury exists in nature primarily as cinnabar (HgS), which is relatively insoluble and not very mobile [60]. Mining waste materials in the New Almaden Mining District have historically included calcines, overburden, and mine tailings. Calcines contain unconverted cinnabar and metacinnabar, Hg(0), and soluble Hg compounds formed during ore retorting [42]. During high-intensity flow events, mercury-laden sediments are mobilized from mining wastes and transported into creeks and reservoirs [48]. Colloidal transport is an important mechanism in mercury mobilization, because most mercury is mobilized in the particulate form, bound to sediments [41]. Guadalupe Reservoir is thought to have been built on former mercury processing area containing waste material [4].

Sulfate-reducing bacteria are known to primarily methylate Hg(II), which is water-soluble and potentially reactive [47]. Since the predominant forms of mercury in mining waste are elemental Hg(0) and unroasted cinnabar (HgS), disollution and oxidation to Hg(II) are thought to limit the concentration of mercury available for methylation [24]. Mercury can be oxidized in various ways. Cinnabar in waste material is more soluble in acidic conditions, which could enhance the oxidation [43]. Dissolved organic matter can dramatically enhance mercury release from cinnabar, possibly through surface complexation [64]. When acid mine drainage is present, such as at the New Idria Mercury Mine in San Benito County, ferric iron can oxidize cinnabar and form soluble compounds in the presence of chloride ion [16]. Oxidized mercury in the upper Guadalupe River Watershed was likely produced through photooxidation, interactions with dissolved organic carbon, and historically through ore roasting.

Figure 14: Total Mercury Concentrations of Reservoirs and Lake

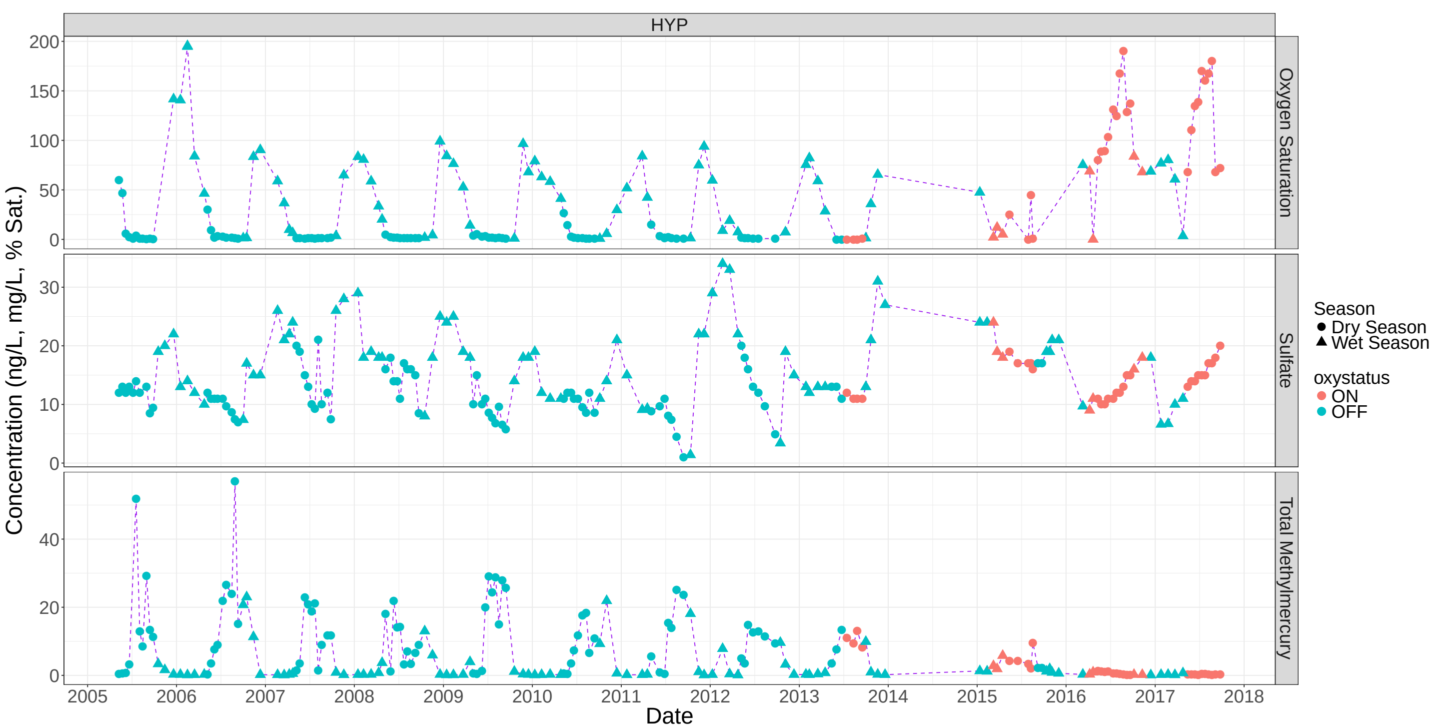


Monitoring Results and Discussion Figure 14 shows epilimnetic and hypolimnetic total mercury concentrations measured during the wet and dry seasons. Epilimnetic total mercury concentrations were higher during the wet season in Almaden ($p<0.001$), Calero ($p<0.01$), Guadalupe ($p<0.05$), and Stevens Creek ($p<0.05$) reservoirs than during the dry season. In Almaden Reservoir, hypolimnetic total mercury concentrations were also higher during the wet season ($p<0.001$). This suggests mercury loading with reservoir inflow during periods of precipitation. High flow events transport the largest loads of mercury to reservoirs, primarily bound to sediments. Additionally, turbulence due to inflows may mobilize mercury already present in reservoirs. In Stevens Creek Reservoir, atmospheric deposition and natural soil erosion are likely accelerated with winter precipitation. Over 50% of Stevens Creek Reservoir's catchment area contains the Franciscan Formation geologic unit responsible for the cinnabar at New Almaden, so soils may be naturally elevated in mercury. Almaden Lake exhibits no significant seasonal variation in total mercury concentrations. This may be a result of constant mercury loading through mining wastes still present in Los Alamitos Creek and its tributaries. Total mercury concentrations were significantly higher in the hypolimia of the reservoirs ($p<0.001$) and Almaden Lake ($p<0.01$) than in the epilimnia, reflecting accumulation in bottom-sediments.

Almaden Lake and Guadalupe reservoir contained statistically similar total mercury concentrations that were the highest measured, with median hypolimnetic concentrations of 26.5 ng/L (SD=62.8) and 30 ng/L (SD=21.9), respectively. Almaden Lake receives inflow from Calero and Almaden Reservoirs through Los Alamitos Creek, which hosts legacy calcine deposits. Unique of the water bodies studied, the lake also captures urban runoff. Guadalupe Reservoir is located downslope from various mines at New Almaden, and is thought to be built on a former mercury processing yard. Receiving contaminated inflow primarily from Jacques Gulch, Almaden Reservoir contained lower total mercury concentrations than Almaden Lake and Guadalupe Reservoir. While hypolimnetic total mercury concentrations are higher in Stevens Creek Reservoir than in Calero Reservoir, epilimnetic concentrations are lower. This could reflect the effects of water deliveries from San Luis and Almaden reservoirs. Stevens Creek Reservoir is located 1.4 miles from the Lehigh Hanson Permanente Cement Plant. A study published in 2010 found elevated wet deposition of mercury in the vicinity of the cement plant, likely supplying mercury to Stevens Creek Reservoir [67].

7.13 Total Methylmercury

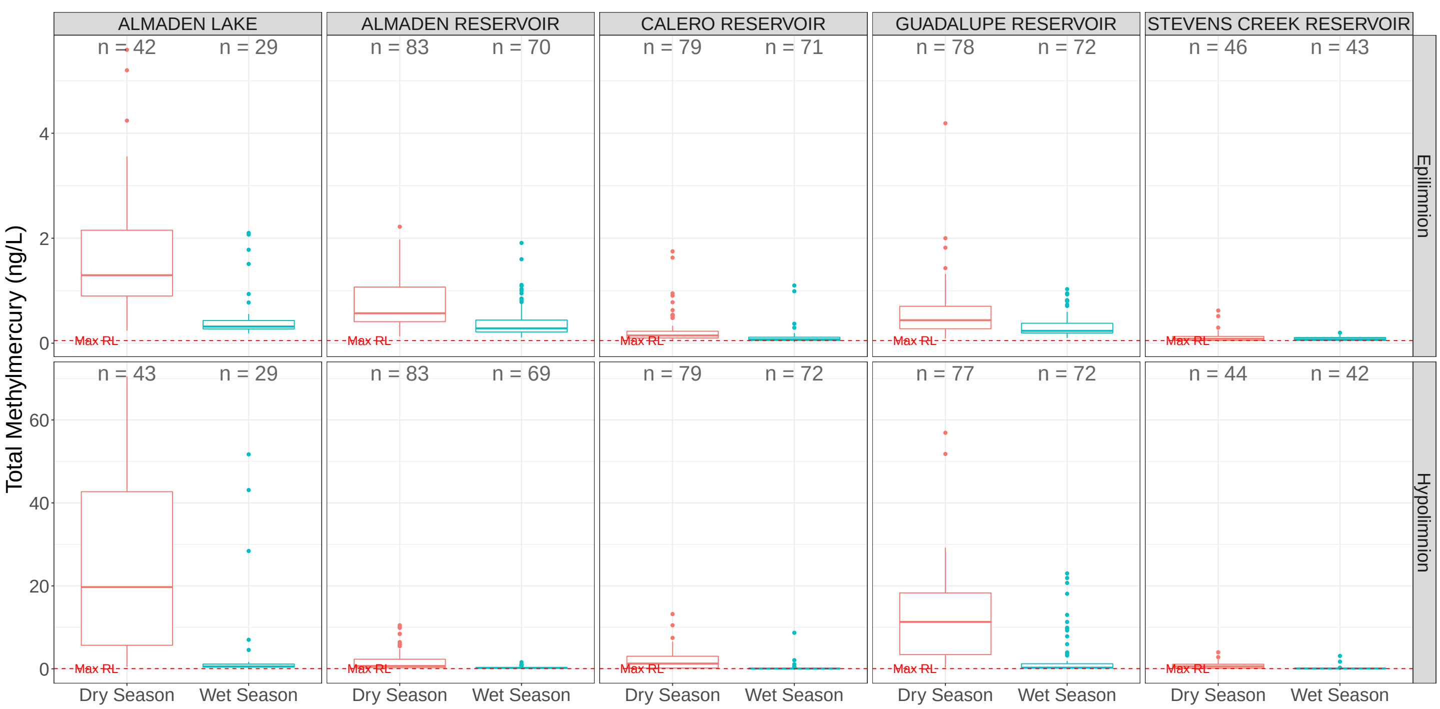
Figure 15: Typical Correspondence of Hypolimnetic Anoxia, Sulfate Reduction, and Methylmercury Production: Guadalupe Reservoir



Each reservoir exhibits seasonal patterns of oxygen depletion, sulfate reduction, and methylmercury production (Figure 15). Following the onset of stratification, the hypolimnion is physically isolated from atmospheric and photosynthetic oxygen sources. Aerobic bacteria degrade organic matter, consuming oxygen in the process. When redox potential declines to approximately 0 to -150 mV, anaerobic sulfate-reducing bacteria metabolize sulfate, producing sulfide, and decreasing sulfate concentrations in bottom waters. These summer sulfate and oxygen minima coincide with peaks in methylmercury, which is produced incidentally as a byproduct of sulfate reduction.

Though sulfate-reducing bacteria are thought to be the primary microbes responsible for methylmercury production, a variety of bacteria can methylate Hg(II), all of which are obligatory anaerobes. These include iron reducers, methanogens, and Firmicutes containing the *hgcAB* gene cluster necessary for methylation [39]. Furthermore, anaerobic sulfurization of dissolved organic matter can increase the bioavailability of mercury for methylation by increasing aggregation and solubility [40]. Microbial methylation occurs predominantly in anaerobic sediments and bottom waters, but also can occur in oxic water columns of freshwater systems [27]. Methylmercury enters phytoplankton through passive diffusion, introducing the compound to the food web. Following algal uptake, methylmercury concentrates increasingly as it moves up the food chain to zooplankton, prey fish, and predatory fish. The largest biomagnification of methylmercury occurs between the water column and algae (a 100,000-fold increase). Thus, reducing aqueous methylmercury concentrations may be the most efficient means of reducing bioaccumulation of the compound.

Figure 16: Total Methylmercury Concentrations of Reservoirs and Lake



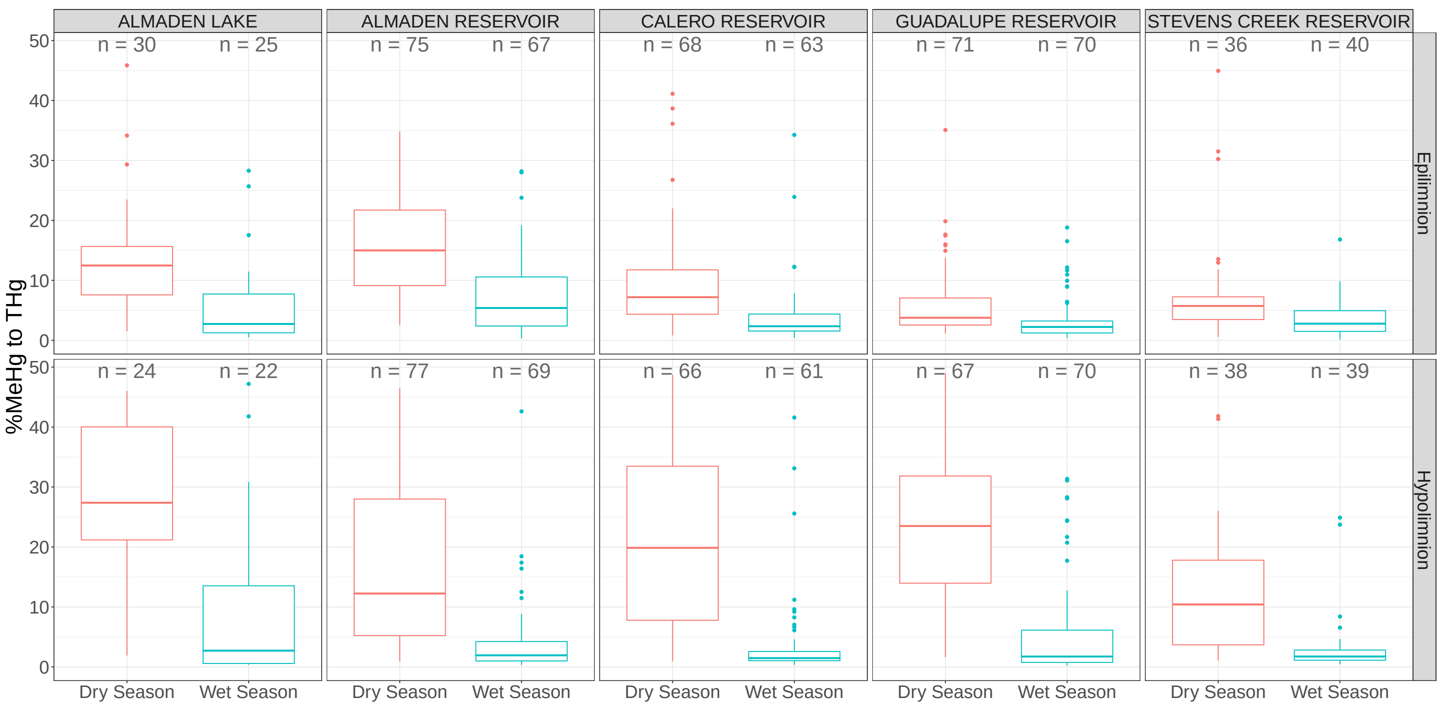
Monitoring Results and Discussion Figure 16 shows total methylmercury concentrations measured during the dry and wet seasons in the epilimnia and hypolimnia of the reservoirs and lake. Total methylmercury concentrations were significantly higher during the dry season than during the wet season ($p<0.001$), illustrating periodic summer production coinciding with hypolimnetic hypoxia. Total methylmercury concentrations were significantly higher in the hypolimnia than in the epilimnia of the water bodies($p<0.001$), indicating production and efflux from the sediment-water interface.

Almaden Lake contained the highest hypolimnetic total methylmercury concentrations, with a median concentration of 19.7 ng/L (SD=21.6) during the dry season, and peaks as high as 70.5 ng/L. Methylmercury concentrations were lower in Guadalupe Reservoir's hypolimnion, with a median concentration of 11.3 ng/L (SD=11.1) and peaks as high as 57 ng/L. Almaden Reservoir's hypolimnetic total methylmercury levels were surprisingly low for a mining-impacted reservoir, with concentrations that were statistically similar to Stevens Creek Reservoir. However, peak concentrations were 6.5 ng/L higher in Almaden Reservoir, reaching 10.5 ng/L. Almaden Lake contained the highest epilimnetic total methylmercury concentrations, with a median concentration of 1.3 ng/L (SD=1.23) during the dry season, and peaks as high as 5.6 ng/L. Dry season epilimnetic methylmercury concentrations were lower and statistically similar in Almaden and Guadalupe reservoirs, with median concentrations of 0.57 ng/L (SD=0.49) and 0.44 ng/L (SD=0.56), respectively. Calero and Stevens Creek Reservoir contained statistically similar epilimnetic methylmercury concentrations during the dry season, with median values of 0.15 ng/L (SD=0.3) and 0.09 ng/L (SD=0.11), respectively.

7.14 Methylation Efficiency

In the absence of empirically determined methylation rates, the percentage of methylmercury to total mercury concentration can describe the efficiency at which an aquatic environment converts mercury to methylmercury. This ratio varies based on hydrological, chemical, climatological, and spatial factors. Various studies have used this metric to evaluate methylation risk between habitat types [37].

Figure 17: Percent TMeHg to THg Concentrations of Reservoirs and Lake



Monitoring Results and Discussion Figure 17 shows the percentages of total methylmercury to total mercury concentrations (methylation efficiency) measured during the dry and wet seasons in the epilimia and hypolimnia of the reservoirs and lake (untreated conditions). Methylation efficiency was significantly greater during the dry season in the epilimnia and hypolimnia of the water bodies ($p<0.001$), supporting the proposed mechanism of production in anoxic hypolimnia during thermal stratification. Methylation efficiency was significantly higher in the hypolimnia of Calero ($p<0.001$), Guadalupe ($p<0.001$), and Stevens Creek ($p<0.05$) reservoirs, and Almaden Lake ($p<0.001$) than in the epilimnia during the dry season. This suggests that the majority of methylmercury production occurs at the sediment-water interface in these reservoirs.

Unique to the water bodies studied, Almaden Reservoir exhibited no significant difference in dry-season methylation efficiency between its epilimnion and hypolimnion. This could be due to enhanced vertical entrainment of methylmercury from its hypolimnion, or to amplified methylmercury production in the reservoir's water column. Almaden Reservoir's low mean depth may support vertical entrainment of profundal compounds into the photic zone. A 2016 study found that settling particles in a freshwater lake contained ten-fold greater methylmercury concentrations than profundal sediments [27]. Almaden Reservoir may experience enhanced methylmercury production in its water column due to the erosive nature of its catchment area, which largely consists of Franciscan Formation soils naturally enriched with mercury (though low in concentration when compared to mining wastes). Littoral and marginal sediments could support methylmercury production and efflux into surface waters. Almaden Reservoir's relatively high area of connected wetlands could also introduce methylmercury into the photic zone.

Increased duration of anoxia and trophic state positively correlate with methylation efficiency. Dry season hypolimnetic methylation efficiency was highest in Almaden Lake, statistically similar in Calero and Guadalupe reservoirs, and lowest in Almaden and Stevens Creek Reservoirs (at α=0.05 significance level). In the epilimnia, dry season methylation efficiency was significantly higher in Almaden Lake and Almaden Reservoir than in the other water bodies. This suggests that these water bodies experience enhanced methylation in their water columns, or that profundal compounds are transported to the epilimnion more readily during the dry season. Because methylmercury enters the aquatic food web in the photic zone through passive diffusion into phytoplankton, mercury in these water bodies may be more bioavailable. Almaden Lake's higher methylation efficiency may be due to its prolonged period of anoxia resulting from its high nutrient concentrations and enriched trophic status. This is evident by abundant biomass containing chlorophyll *a* and phycocyanin and enhanced internal loading of nutrients from bottom sediments during periods of stratification.

7.15 Food Web

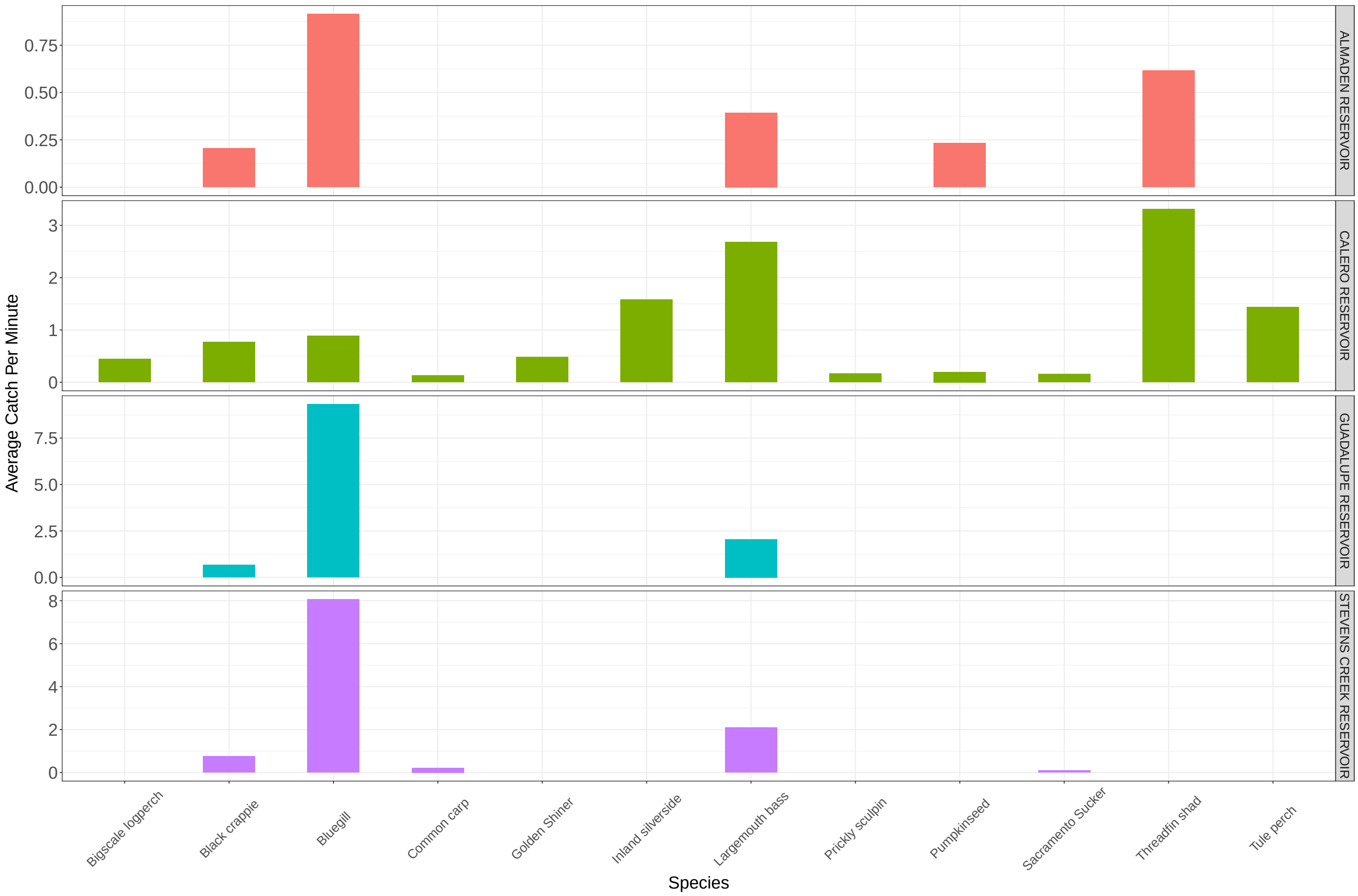
Trophic structure and dietary patterns are major factors controlling bioaccumulation in freshwater ecosystems. Trophic position and growth rate are significant determiners of methylmercury concentration, with top predators and slow-growing fishes containing elevated mercury concentrations [5] [76]. Studies investigating the relationship between stable isotope ratios of nitrogen and mercury concentration confirmed increased biomagnification with trophic level [51]. Food web length has a significant effect on mercury concentrations in top predators. Lakes containing abundant pelagic crustaceans and forage fish have been documented to contain higher mercury concentrations, likely due to these species filling crucial linkages between predatory fish and zooplankton [17]. Furhtermore, studies document higher methylmercury concentrations in the pelagic-based food web than those that occur in the benthic-based foodweb [72].

Monitoring Results and Discussion

Almaden Reservoir In recent years, low water levels have encumbered the use of boat electrofishing sampling methods in Almaden Reservoir. Though hook-and-line sampling was employed as an alternative, it introduces considerable bias toward larger, more predatory fish. As a result, the following results may present less certainty than the other reservoirs. Almaden Reservoir exhibits more diversity than Guadalupe Reservoir, with pumpkinseed (*Lepomis gibbosus*) and threadfin shad (*Dorosoma petenense*) observed in addition to largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), and black crappie (*Pomoxis nigromaculatus*)(Figure 18). Its longer food web provides an additional trophic level between prey and top predators [1]. The presence of pelagic forage fish may enhance growth rates in predators.

Calero Reservoir Calero Reservoir contains the highest diversity in fish assemblage, with abundant pelagic forage fish (inland silverside, golden shiner, and threadfin shad), sunfish (Centrarchidae family including largemouth bass, bluegill, and black crappie), littoral fish (tule perch), and bottom-dwelling fish (common carp, Sacramento sucker, brown and white bullhead) (Figure 18). Calero Reservoir hosts the longest food web, with a difference of 1.1 trophic units between planktivorous prey and top predators, potentially enhancing bioaccumulation [1]. However, the presence of fish species occupying a wide range of trophic positions could contribute to enhanced growth rates in predators, perhaps diluting methylmercury concentrations through somatic growth dilution.

Figure 18: Average Catch Per Minute of Predominant Reservoir Fish Species



Guadalupe Reservoir Guadalupe Reservoir hosts low fish diversity, with the predominant species observed consisting of largemouth bass, bluegill, and black crappie (Figure 18). These species constitute a short food web, with a difference of three-fifths of one trophic position [1].

Almaden Lake Biological monitoring in Almaden Lake is accomplished by the Coordinated Monitoring Program, which completed fish sampling in 2012, 2013, and 2016. The consultant, AECOM, used a combination of methods, including backpack electrofishing and seine netting, to gather samples for body burden mercury analysis. Though ascertaining the lake's fish assemblage was not the objective of this effort, and results are not likely to represent true population dynamics, AECOM noted species observed in 2016. Largemouth bass comprised 86% of species observed, with one to two of each bluegill, mosquitofish, prickly sculpin, inland silverside, and Sacramento sucker. These results represent a gap of 0.7 trophic positions between top predators and prey fish observed [1].

Reference Site Stevens Creek Reservoir exhibits a similar assemblage as Guadalupe Reservoir, with largemouth bass, bluegill, and black crappie comprising the majority of species observed (Figure 18). This short food web may contribute to decreased bioaccumulation in top predators.

Discussion Guadalupe and Stevens Creek reservoirs contain short food webs, with no true prey fish observed. While their minimal trophic diversity may result in decreased bioaccumulation, growth rates in predators may be slower in the absence of prey, potentially restricting somatic growth dilution. Also worth noting is that predatory fish will resort to cannibalism in the absence of prey. In this circumstance, juveniles of the predatory species fill the trophic positions of the smaller forage species, creating similar foodweb lengths as more complex systems despite the apparent lack of diversity.

Almaden Reservoir's slightly longer food chain, owing to the presence of threadfin shad, may promote enhanced trophic transfer of methylmercury between zooplankton and predatory fish. Calero Reservoir's diverse fish assemblage with abundant prey fish may increase bioaccumulation through enhanced trophic-transfer, but could also decrease concentrations in predators if prey abundance augments fish growth. Almaden Lake's fish assemblage may be consistent with those of Guadalupe and Stevens Creek reservoirs, but sampling bias confounds interpretations. The lake's connection to Los Alamitos Creek may cause it to experience decreased bioaccumulation, as fish are not confined to the impoundment. However, summer creek dry-back may restrict fish relocation, forcing fish to remain in Almaden Lake when methylmercury production is high.

7.16 Bioaccumulation Factors

Bioaccumulation factors (BAFs) describe the degree of biomagnification that occurs between the water column and biota. Variation in bioaccumulation efficiency results from the chemical, biological, physical, and operational differences of the reservoirs. This section compares mercury concentrations (mg/kg, wet weight) in 100 mm length-standardized largemouth bass (n=5 to n=25) to the average aqueous methylmercury concentration (ng/L, n=8 to n=15) measured over its life span in each lake compartment, with age inferred based on fork length and season collected. Apart from Almaden Lake, bioaccumulation factors were calculated from data collected before reservoir treatment began.

Table 5: Bioaccumulation Factors of Reservoirs and Lake between Hypolimnion and 100 mm Bass

Reservoir	Min BAF (District)	Max BAF (District)	BAF (TMDL)
Almaden Reservoir	3.93×10^5	1.2×10^6	2.20×10^5
Calero Reservoir	1.8×10^5	4.5×10^5	7.6×10^4
Guadalupe Reservoir	1.13×10^5	1.95×10^5	1.50×10^5
Stevens Creek Reservoir	3.0×10^5	4.8×10^5	NA
Almaden Lake	6.7×10^4	1.72×10^5	NA

Table 6: Bioaccumulation Factors of Reservoirs and Lake between Epilimnion and 100 mm Bass

Reservoir	Min BAF (District)	Max BAF (District)
Almaden Reservoir	1.05×10^6	2.07×10^6
Calero Reservoir	1.8×10^6	3.2×10^6
Guadalupe Reservoir	2.5×10^6	4.5×10^6
Stevens Creek Reservoir	1.5×10^6	3.3×10^6
Almaden Lake	4.84×10^5	6.86×10^5

Results and Discussion The Guadalupe River Watershed TMDL Staff Report published BAFs comparing average hypolimnetic total methylmercury and mercury in age-1 largemouth bass using data collected from 2003 to 2004 in Almaden, Calero, and Guadalupe reservoirs. It reported multipliers of 220,000, 76,000, and 150,000, respectively [4]. While the BAF reported for Guadalupe Reservoir fell within the range of this analysis, new data suggest much higher BAFs occurring in Almaden and Calero reservoirs (Table 5).

Because methylmercury is introduced into the food web by passive diffusion into phytoplankton, methylmercury concentrations in epilimnetic waters are better representative of the compound's availability for biological uptake. Table 6 shows bioaccumulation factors calculated between surface waters and bass. Almaden Lake experiences the smallest bioaccumulation factor between surface waters and 100 mm bass. This is likely due to bloom dilution resulting from its enhanced trophic status, its short food web, and lack of a confining structure (which may allow fish to move to and from the impoundment). Almaden Reservoir, with less primary productivity and a greater population of pelagic forage fish, experiences a higher degree of bioaccumulation. Calero and Stevens Creek reservoirs host similarly high BAFs. Though Calero Reservoir is considerably more eutrophic, its long food web may counteract the effects of bloom dilution. Guadalupe Reservoir, which is relatively oligotrophic and hosts a short food web, experiences the most pronounced level of biomagnification.

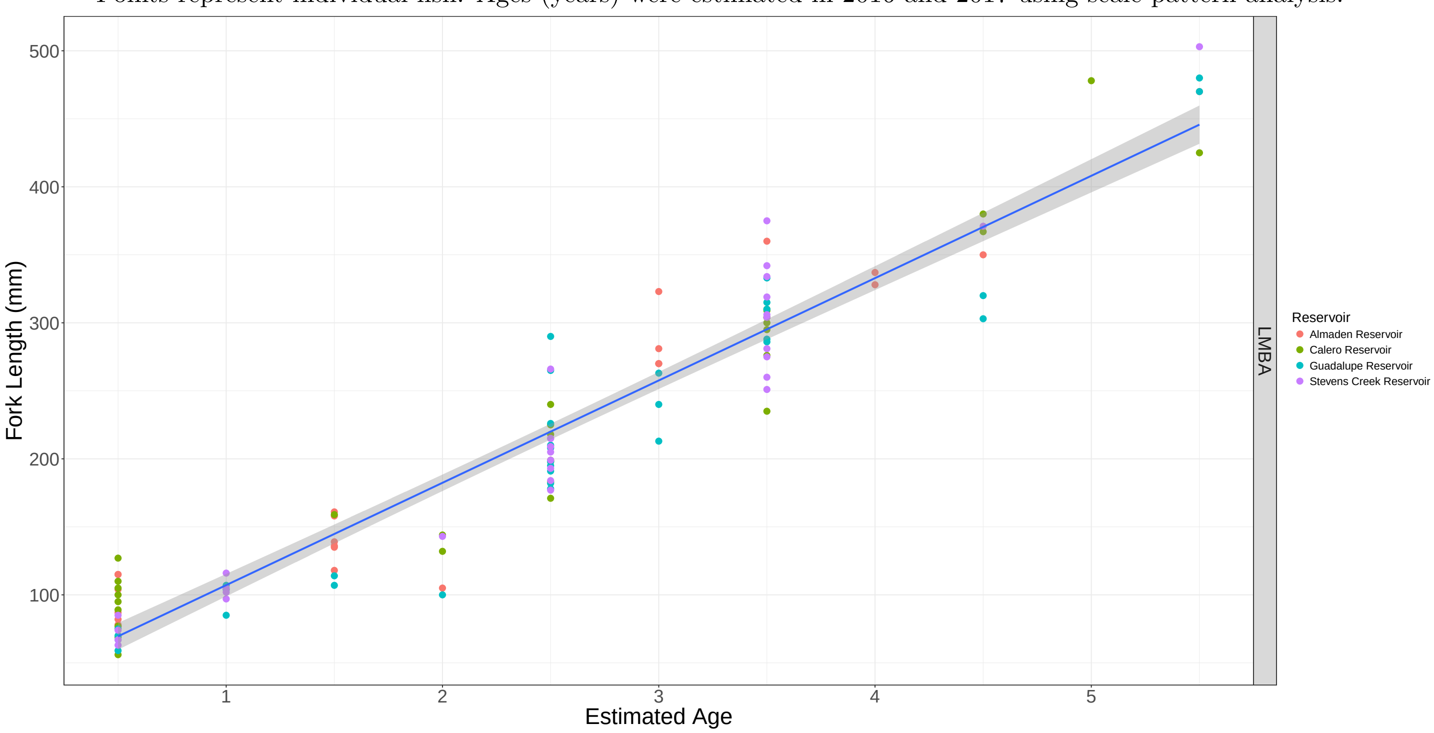
7.17 Fish Growth Rates

Somatic growth dilution occurs when organisms experience a greater proportional gain in mass relative to toxicant concentration. The presence of a high-quality food source can cause biota to grow rapidly, reducing accumulation and trophic transfer of methylmercury. Somatic growth dilution can occur in any trophic level of the foodweb, from zooplankton to predatory fish. One study suggested that high phosphorus-to-carbon algae increased the growth rate of *Daphnia* by 3.5-fold, dramatically reducing their methylmercury concentration [49]. Growth rates of zooplankton could explain some variation in fish tissue mercury concentrations in lakes of differing trophic statuses. Another study demonstrated that individual growth rate accounted for 38% of the variation in mercury concentrations measured in stream-dwelling Atlantic salmon [78]. Therefore, growth rates of organisms throughout the food web can influence mercury concentrations in predatory fish.

This section reports site-specific fish growth rates to elucidate the role of somatic growth dilution in influencing the extent of bioaccumulation supported by each reservoir. Fish ages were estimated using scale pattern analysis [52]. While this method is best suited for non-temperate regions that experience predictable freeze and thaw cycles, it is a useful tool for deducing fish age to estimate growth rates.

Figure 19: Length to Age Relationship of Largemouth Bass

Points represent individual fish. Ages (years) were estimated in 2016 and 2017 using scale pattern analysis.

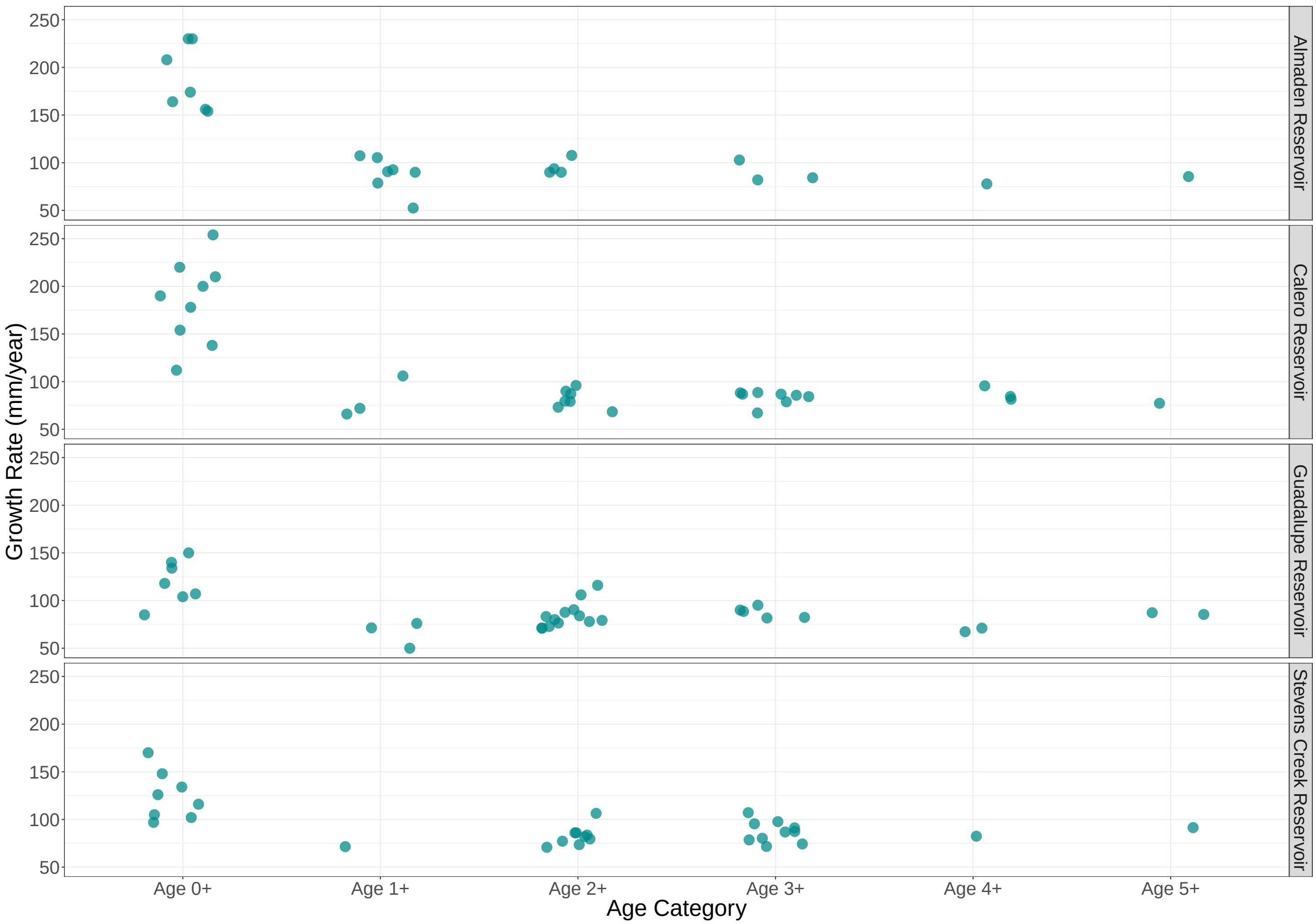


Results: Length to Age Relationship Figure 19 shows the relationship between length and age measured for largemouth bass. Age 0+ includes fish aged as 0.5 to 1 year, and age 1+ includes fish aged as 1.5 to 2 years.

Age 0+, or young-of-year, largemouth bass (n=31) ranged from 56 mm to 127 mm, with an average length of 89 mm (95% CI= 82, 96). This is reasonably consistent with the 55-102 mm range referenced for age-1 largemouth bass in the Guadalupe River Watershed during the development of the TMDL.

Figure 20: Reservoir-Specific Growth Rates of Largemouth Bass

Points represent individual fish. Ages were estimated in 2016 and 2017 using scale pattern analysis.



Results: Guadalupe River Watershed and Reference Site Figure 20 shows reservoir-specific growth rates of largemouth bass. Typically, fish experience the most pronounced growth during their first year, which decreases at age 1 and remains relatively constant throughout the rest of their lives. Age 0+ largemouth bass in Almaden Reservoir (n=7) exhibit an average growth rate of 188 mm per year (95% CI= 163, 213). Since fish do not reach these lengths by age 1, this indicates that growth slows following their initial half-year. Therefore, collection season is an important factor in interpreting mercury body burden results in young-of-year fish. Age 0+ largemouth bass in Calero Reservoir (n=9) exhibit an average growth rate of 184 mm per year (95% CI= 155, 213). Age 0+ largemouth bass in Guadalupe Reservoir (n=7) exhibit an average growth rate of 119 mm per year (95% CI= 102, 136). Age 0+ largemouth bass in Stevens Creek Reservoir (n=8) exhibit an average growth rate of 125 mm per year (95% CI= 108, 142). The growth rates of age 0+ largemouth bass are significantly higher in Almaden and Calero Reservoirs than in Guadalupe and Stevens Creek Reservoirs ($p<0.001$). There is no significant difference in growth rates of largemouth bass age 1+ or greater between reservoirs.

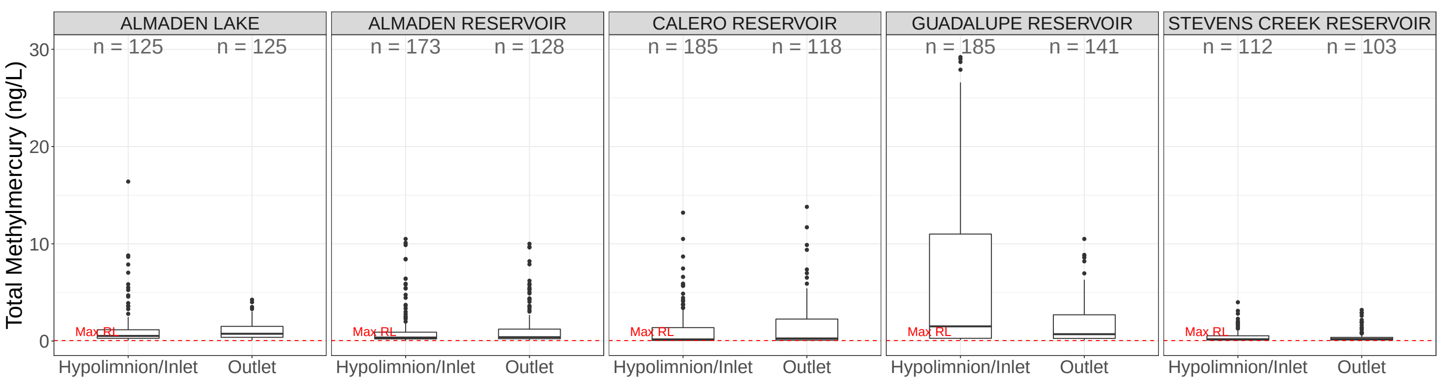
Discussion Juvenile largemouth bass consume zooplankton, rotifers, and crustaceans before transitioning to insects and fish fry at 50-60 mm in length, and primarily to fish at 100-125 mm in length [58]. Diet patterns of adult largemouth bass are variable, as they can change foraging behavior depending on food availability.

In Calero and Almaden reservoirs, which contain populations of pelagic forage fish, growth rates of juvenile largemouth bass are higher than in Guadalupe and Stevens Creek reservoirs, which do not appear to support true prey species. Almaden and Calero Reservoirs contain higher concentrations of Chlorophyll *a*, which likely support larger populations of zooplankton and lower trophic level fish. In Guadalupe and Stevens Creek Reservoirs, young largemouth bass diets are likely to be more reliant on crustaceans, insects, and cannibalism, which may restrict their growth. Adult largemouth bass likely feed on bluegill and black crappie. The higher growth rate of age 0+ largemouth bass in Almaden and Calero reservoirs may reduce mercury concentrations through somatic growth dilution, potentially elucidating the higher bioaccumulation factors observed in Guadalupe Reservoir (Table 6).

7.18 Outlet and Hypolimnetic Methylmercury Concentrations

Hypolimnetic methylmercury samples are taken adjacent to the outlet intakes at the deepest location in the reservoirs. These areas experience sustained hypoxia during summer stratification, and are likely to support the highest production of methylmercury at the sediment-water interface [79]. Due to their proximity to the penstock outlets, one would expect hypolimnetic methylmercury concentrations to be consistent with concentrations measured at the reservoir outlets. Therefore, methylmercury concentrations measured in the hypolimnion may represent a reliable proxy for estimating concentrations discharged downstream, or vice versa.

Figure 21: Comparison of Hypolimnion (Reservoirs) or Inlet (Lake) to Outlet Methylmercury Concentrations



Results: Guadalupe River Watershed

Reservoirs Almaden and Calero reservoirs exhibit statistically similar methylmercury concentrations measured between their hypolimnia and outlet works (Figure 21). The outlet intakes of these reservoirs are contiguous with the reservoir bottoms, discharging hypolimnetic waters downstream. Unlike the other reservoirs, Guadalupe Reservoir's outlet intake extends approximately three meters above the reservoir bottom. This creates a stagnant hypolimnetic pool that contributes to the reservoir's prolonged period of stratification. Methylmercury concentrations measured at Guadalupe Reservoir's outlet are significantly lower than concentrations measured in its hypolimnion ($p<0.001$), because methylmercury primarily accumulates near the sediment-water interface, and this is where water samples are collected (1 meter above sediment-water interface)(Figure 21).

Almaden Lake In previous reports, Almaden Lake was thought to be a sink for methylmercury. However, this was likely due to the prior inlet sampling site being located within the lake, where stagnation and backflow occurred. In May of 2016, the sampling site was moved to the concrete weir in Alamitos Creek, under the pedestrian bridge south of Almaden Lake (Appendix A.8). Recent findings have confirmed the lake as a source of methylmercury to downstream waters, with significantly higher methylmercury concentrations measured at its outlet than its inlet ($p<0.001$)(Figure 21). There is no significant difference between aqueous total mercury concentrations measured at the lake's inlet and outlet, suggesting that the impoundment's presence does not reduce inorganic mercury loading downstream. However, during large flow events, high-mercury sediments may accumulate in the lake.

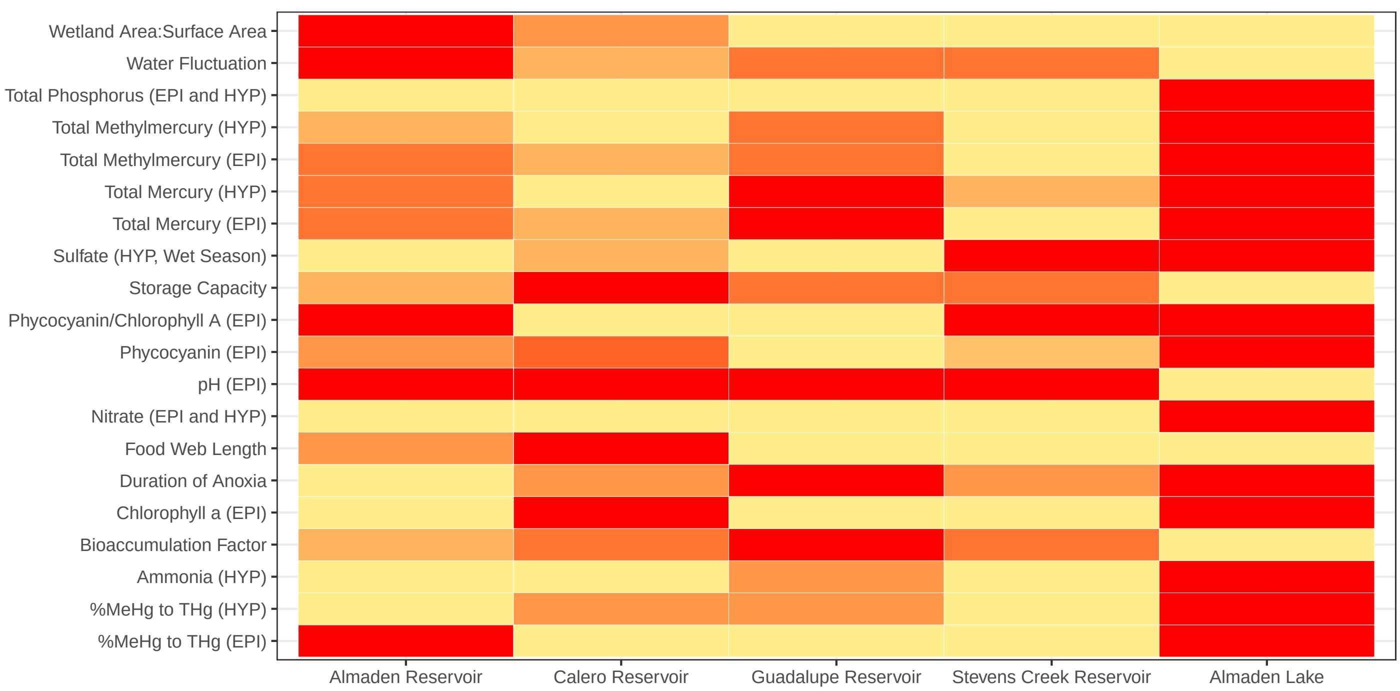
Results: Reference Site Stevens Creek Reservoir's outlet is configured in a similar manner to those of Almaden and Calero reservoirs, with the penstock intake laying contiguous to the reservoir's bottom. There is no significant difference between total methylmercury concentrations measured between its hypolimnion and at its outlet (Figure 21).

7.19 Summary: Comparative Study

The four impaired water bodies included in the Guadalupe Watershed Mercury TMDL and the reference reservoir exhibit various hydrological, chemical, biological, and infrastructural differences and similarities. These characteristics are summarized in figure 22. Methylation efficiency generally increases with length of stratification and trophic status, but bioaccumulation is reduced in eutrophic lakes and reservoirs. Abundant algal biomass available for decomposition by aerobic heterotrophs compounds seasonal anoxia, creating ideal conditions for the proliferation of sulfate-reducing bacteria. However, increased algae content reduces bioaccumulation through bloom dilution.

Figure 22: Summary of Comparative Study

Red tiles reflect relatively high values measured, while yellow tiles reflect low values.



Almaden Reservoir Almaden Reservoir is the smallest reservoir included in this study, and experiences dramatic water level fluctuations due to its small storage capacity. It is mesotrophic, experiencing little internal nutrient loading due to its short period of stratification, and minimal external nutrient loading due to its rural, upland location. Having received mining waste directly, it contains relatively high total mercury concentrations, though not as high as Almaden Lake or Guadalupe Reservoir. It has relatively low methylmercury concentrations and methylation efficiency in its hypolimnion, but relatively high methylmercury concentrations in its epilimnion.

This may enhance the compound's availability at the base of the food web. Almaden Reservoir contains the highest area of connected wetlands, which may be zones of methylation. Its moderate food web length may enhance bioaccumulation, but relatively rapid growth rates in bass may support somatic growth dilution.

Calero Reservoir Calero Reservoir is the largest reservoir included in this study, experiencing the smallest water level fluctuation due to its large size and mainly imported water supply. It has a high surface area relative to its depth, causing weak thermal stratification that may facilitate vertical entrainment of profundal compounds into the photic zone. Internal nutrient loading during periods of stratification and external nutrient loading from upstream agricultural land may contribute to its relatively high algal productivity. Calero Reservoir is contaminated periodically by water transfers from Almaden Reservoir through the Almaden-Calero Canal, causing it to possess relatively low total mercury concentrations. However, its eutrophic status creates optimal conditions for methylation, contributing to its moderately high methylation efficiency. Though its long food web may enhance the trophic transfer and biomagnification of mercury, its high algae concentrations likely reduce methylmercury in biota through bloom dilution.

Guadalupe Reservoir Guadalupe Reservoir is located immediately downslope of the New Almaden Mining District, has received mercury-laden sediments and waste material directly, and is thought to have been built on a former cinnabar processing yard. As a result, it has among the highest total mercury concentrations measured in the watershed. Its dramatic water level fluctuations may stimulate microbial activity and contribute to its moderately high methylation efficiency. Its low surface area to depth and elevated outlet structure cause the reservoir to experience a prolonged period of stratification and enhanced methylmercury production. However, its stable stratification may serve as a barrier from profundal compounds entering the photic zone during the dry season. Guadalupe Reservoir's minimal internal and external nutrient loading contribute to its mesotrophic status and relatively low algal productivity. While this may reduce the extent of bloom dilution that occurs, its short food web may decrease trophic transfer and biomagnification of methylmercury.

Almaden Lake Almaden Lake is a relatively small, shallow impoundment along Los Alamitos Creek. It is highly eutrophic, experiencing extensive external nutrient loading due to its urban land use, and internal nutrient loading resulting from its long period of stratification. It contains similar total mercury concentrations to Guadalupe Reservoir, which are the highest measured in the watershed. Though the District has made considerable progress in removing mining waste material, Los Alamitos Creek hosts abundant calcine deposits, potentially supplying mercury to the lake. Its methylmercury concentrations are the highest in the watershed, and it experiences the greatest methylation efficiency. Despite this, its bioaccumulation factors are notably lower than the other reservoirs, perhaps due to its short food web and high algae concentrations, which may reduce methylmercury concentrations in biota through bloom dilution.

Stevens Creek Reservoir Stevens Creek Reservoir is comparable in size to Guadalupe Reservoir, causing it to experience similar water level fluctuations. Like Guadalupe Reservoir, it hosts a short food web, relatively low nutrient concentrations, and low algal productivity. With most of its mercury contamination presumed to be a result of atmospheric deposition, it contains low total mercury and methylmercury concentrations. Though it contains significantly higher background sulfate concentrations than other reservoirs, this excess sulfate does not appear to stimulate methylation by sulfate-reducing bacteria. Stevens Creek Reservoir has the lowest epilimnetic and hypolimnetic methylation efficiency, but its fish experience a moderate degree of bioaccumulation. This may be a result of its short food web, low potential for bloom dilution, and stunted growth rates in juvenile largemouth bass.

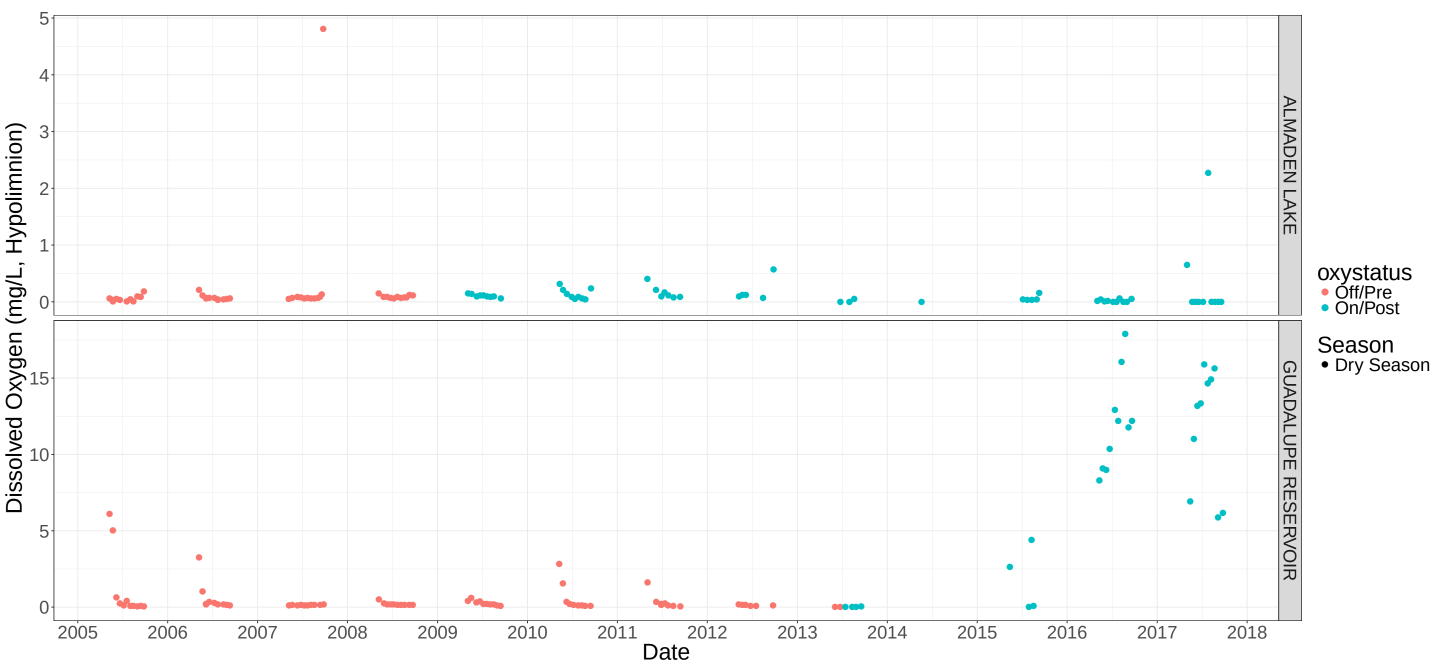
8 Special Study 2: Treatment System Effectiveness

Section 9.10 of the Guadalupe River Watershed TMDL Staff Report presents the following study question:

"*Is it possible to increase the assimilative capacity for methylmercury in reservoirs and lakes? Is it feasible to do so? If it is feasible, does it result in attaining the fish tissue targets?*"

The District addresses Special Study 2 by evaluating the effectiveness of engineered control systems in reducing methylmercury concentrations in the water column and in fish in each water body. These systems may increase assimilative capacity by suppressing anoxic conditions that facilitate the microbial conversion of ionic mercury to methylmercury. Data are analyzed by comparing dry season (May 1 - September 30) water quality results measured before and during the operation of the treatment systems (Figure 23). Because oxygenation system operation was sometimes intermittent due to brief shut downs, the systems were considered to be "on" if they were known to be operated within 30 days before *and* within 30 days following the sample date, and dissolved oxygen concentration was greater than 3 mg/L in the hypolimnion. This correction prevents the possibility of false negative results (dissolved oxygen concentrations would appear artificially inflated when the system was considered to be off, decreasing the observable difference between "on" and "off" values).

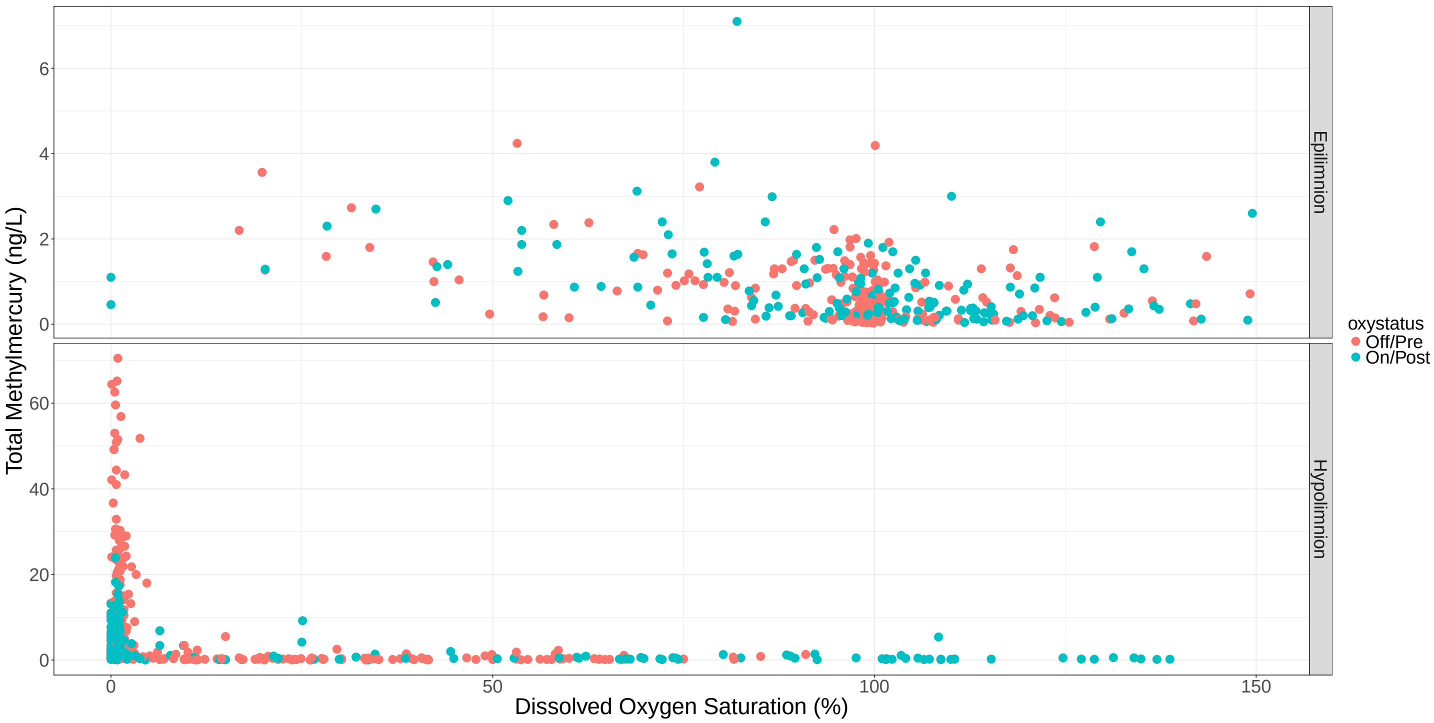
Figure 23: Example of Data Used in Special Study 2 for Lake And Reservoirs



8.1 Theoretical Basis

Various treatment methods have been used to inhibit mercury methylation by poising redox potential in favor of oxidative processes. Aquatic organisms obtain their energy from the redox reactions, and the speciation of elements and compounds commonly found in aquatic environments is often governed by redox potential. Many of these transformations are microbially mediated, and must occur in succession according to their thermodynamic potential, with the strongest oxidant used first [73]. After aerobic heterotrophs consume oxygen, denitrifiers convert nitrate to gaseous nitrogen, then manganese and iron oxides are microbially and abiotically reduced. Following manganese and iron reduction, sulfate-reducers convert sulfate to hydrogen sulfide, and finally methanogens reduce carbon dioxide to methane in the presence of hydrogen [70]. Some management strategies used to inhibit methylmercury production involve poising redox conditions above those which facilitate sulfate reduction by increasing concentrations of compounds used as terminal electron acceptors by non-sulfate reducers. This allows these bacteria to out-compete sulfate-reducing bacteria for energy. Since sulfate- and iron-reducing bacteria, methanogens and firmicutes containing the hgcAB gene cluster have been implicated as mercury methylators, effective inhibition of methylmercury production would poise redox above conditions favorable to iron-reducers [39].

Figure 24: Dissolved Oxygen Saturation and Methylmercury Production: All Water Bodies

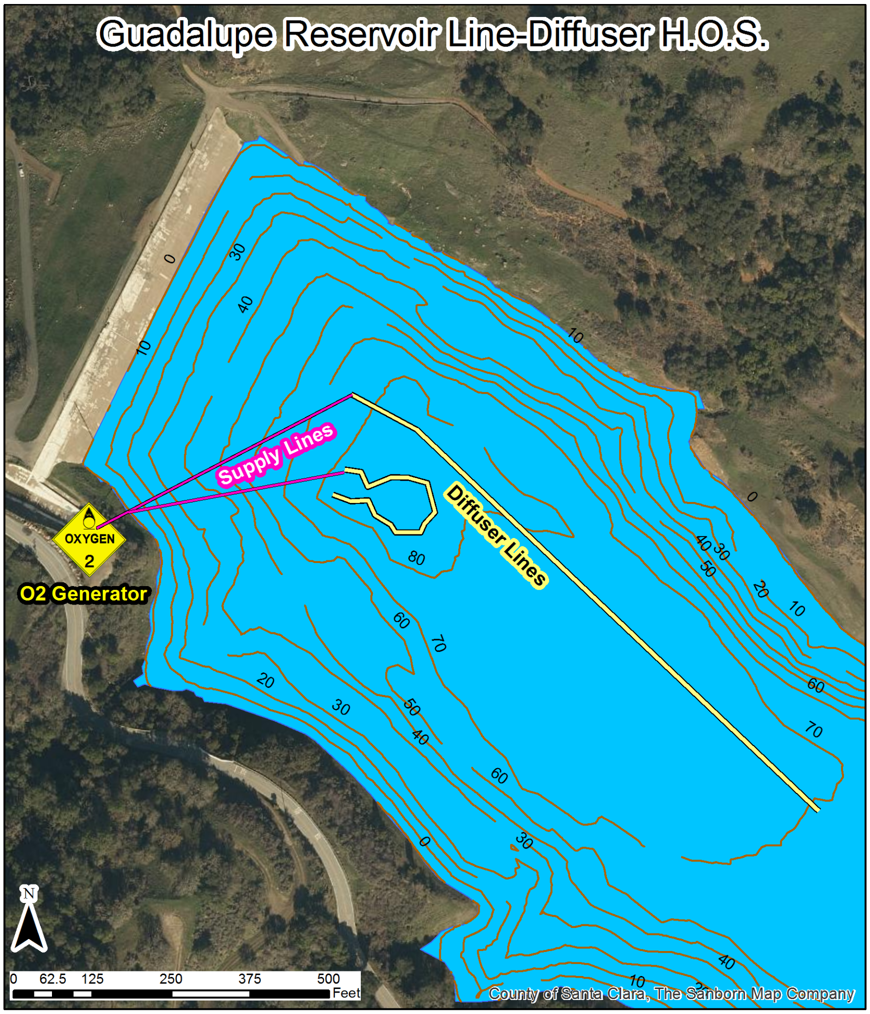


Oxygenation of the sediment-water interface should reduce mercury bioaccumulation by establishing conditions that are unfavorable to the growth of anaerobic bacteria that mediate the conversion of Hg(II) to methylmercury. However, oxygenation may not be effective in reducing methylation in oxic water columns of lakes and reservoirs. Figure 24 shows that hypolimnetic methylmercury production occurs predominantly under low-oxygen conditions, but methylmercury can exist in the epilimnion under a wide range of oxygen concentrations. Methylmercury may be transported to the photic zone from the hypolimnion, or produced in the water column. Littoral sediments may be a source of methylmercury to the epilimnion, as warm sediments with high organic content can become anoxic even when the overlying water does not.

Hypolimnetic Oxygenation Systems The line-diffuser oxygenation systems evaluated in this study inject pure oxygen into the hypolimnia of reservoirs, emitting fine bubbles that dissolve into bottom waters. Oxygen may be stored in tanks, or generated on site. Both options are energy-intensive and costly. The District's hypolimnetic oxygenation systems intake and compress air, filter it to remove nitrogen and other gases, and inject oxygen into the hypolimnia of reservoirs at a designated flow rate. Figure 25 shows the typical system used to oxygenate reservoirs included in this study. The diffuser lines are elevated approximately one foot above the reservoir bottoms, and are designed to deliver 12.5 standard cubic feet of oxygen per minute (0.75 US tons per day). They are situated in deepest areas of the reservoirs, extending up-reservoir from the outlet works. Line-diffuser systems maintain the natural thermal stratification of lakes and reservoirs, providing cold water outlet discharges necessary to maintain compliance with requirements regarding downstream fisheries. When compared to aeration or circulation, hypolimnetic oxygenation results in higher dissolved oxygen levels, less induced oxygen demand, and greater stability of thermal stratification [10].

Various studies have evaluated the use of hypolimnetic oxygenation to improve water quality in lakes and reservoirs. Researchers have reported case studies indicating decreased hypolimnetic phosphorus, ammonia, manganese, and hydrogen sulfide concentrations using hypolimnetic oxygenation [10]. Other studies have shown reduced internal nutrient loading [7] [11] [55]. Hypolimnetic oxygenation has also been shown to reduce iron and

Figure 25: Hypolimnetic Oxygenation System Layout: Guadalupe Reservoir

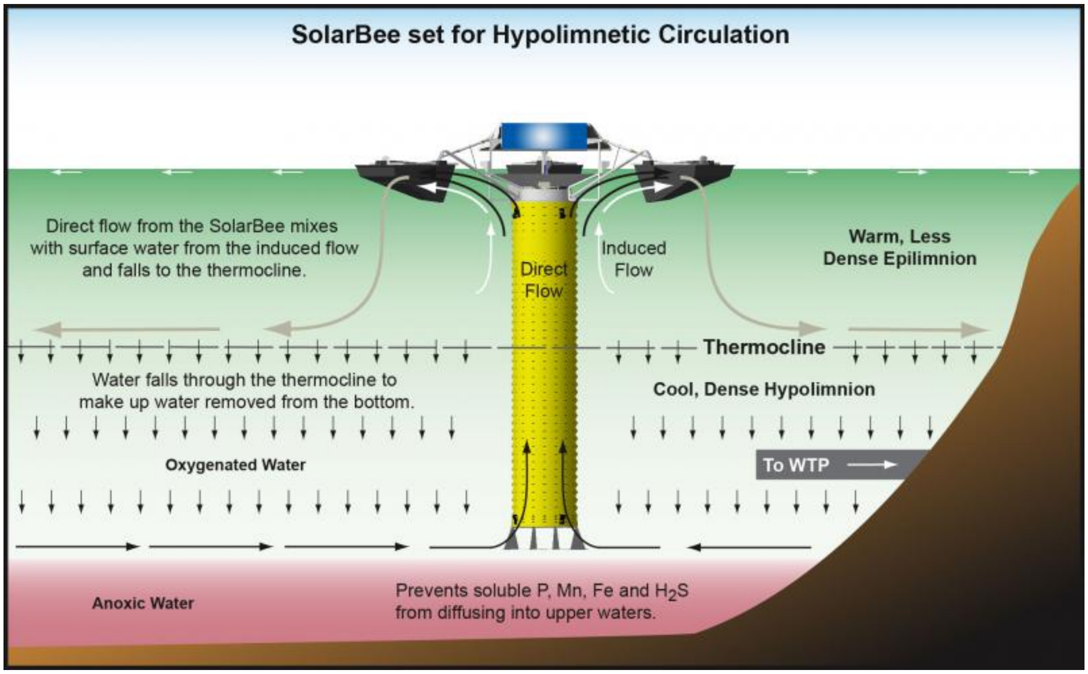


manganese release from reservoir sediments, improving drinking water quality [15]. Hypolimnetic methylmercury concentrations in North Twin Lake, Washington were reduced during oxygenation, but researchers did not observe decreases in mercury in fish tissue or zooplankton [8].

Artificial Circulation Solar-powered circulators are commonly used to improve water quality in lakes and ponds. Epilimnetic circulation intends to create sufficient turbulence in the photic zone to discourage the establishment of phytoplankton and cyanobacteria. Hypolimnetic circulators attempt to improve oxygen concentrations in bottom waters by mixing oxygenated epilimnetic water with oxygen-poor hypolimnetic water, destratifying the water column. Solar-powered circulators are relatively inexpensive, low-maintenance, and require no external power source. Improved dissolved oxygen concentrations in hypolimnetic waters would result in the similar water quality benefits as stated above. Figure 26 shows a schematic produced by SolarBee, illustrating hypolimnetic circulation.

Other Treatment Methods Other methods have been used to reduce methylmercury production in anoxic water and sediments, each attempting to curtail reduction processes through strong oxidant addition. Nitrate addition has been shown to reduce phosphate release from anoxic sediments by curtailing the microbial reduction of iron-oxides to which phosphate is sorbed [11]. It has also been shown to reduce efflux of iron and manganese from sediments [26]. Seasonal maximum methylmercury and soluble reactive phosphorus concentrations in Onandaga Lake, New York decreased 94% and 95% after the application of a whole-lake nitrate plume [56]. Recent studies indicate that fish tissue mercury concentrations in the lake are decreasing. However, nitrate

Figure 26: Solar Circulator Schematic. Image Copyright SolarBee ®



amendments must be applied with caution due to their potential to cause eutrophication. Manganese oxide amendments have been shown to reduce methylmercury production by over 99% in microcosm experiments [77]. Manganese oxide is reduced to Mn(II), which can cause taste and odor issues in drinking water. However, under alkaline conditions Mn(II) would likely precipitate as magnesium carbonate. Activated carbon addition can reduce porewater concentrations and bioaccumulation of methylmercury [39]. The District uses oxygen addition due to its multiple benefits of reducing internal loading of nutrients, metals, and methylmercury combined with its low probability of causing corollary water quality nuisances.

8.2 System Operation

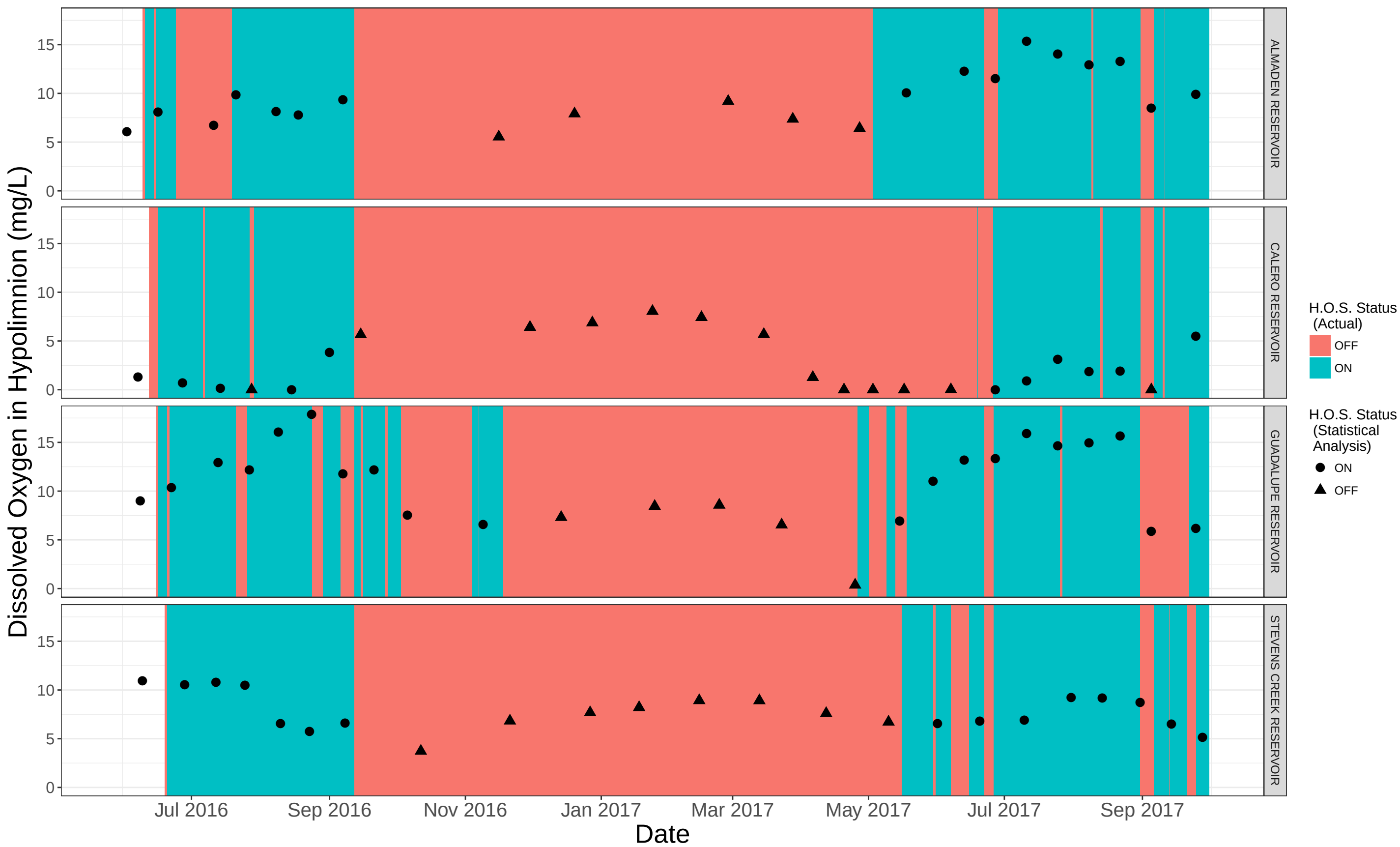
Figure 27 shows records of hypolimnetic oxygenation system operation, as well as statuses considered for the statistical analysis. Because the effects of oxygenation persist during brief shut downs, data collected between operational periods (within one month) when dissolved oxygen concentrations in the hypolimnion remained above 3 mg/L were considered to have an oxygenation system status of "on". Prior to 2016, low water levels due to drought hindered the operation of the systems. Please see previous District Guadalupe River Watershed Mercury TMDL progress reports for details.

Almaden Reservoir Three circulators were deployed in Almaden Reservoir in April of 2007. Two epilimnetic circulators intended to improve planktonic assemblages and reduce load or organic matter to the bottom of the reservoir, while one hypolimnetic circulator aimed to improve oxygen levels and suppress hypoxic conditions that facilitate the methylation of mercury. These systems were found to be ineffective in reducing methylmercury production [28].

In April of 2014, the District installed a hypolimnetic oxygenation system in Almaden Reservoir. This system operated intermittently in 2015, and continuously during periods of thermal stratification occurring in the reporting period of 2016-2017.

Calero Reservoir A hypolimnetic oxygenation system was installed in Calero Reservoir in November of 2011, but was not operated until April of 2013. It operated full-time in 2014, intermittently in 2015, and roughly

Figure 27: Hypolimnetic Oxygenation System Operation: 2016-2017 Reporting Period



continuously during periods of thermal stratification occurring in the reporting period of 2016-2017. There were brief interruptions in operation in summer of 2016, and operation did not begin until anoxia was established in 2017 due to mechanical failure.

Guadalupe Reservoir Three epilimnetic circulators were deployed in Guadalupe Reservoir in 2007 to improve planktonic assemblages and reduce organic loading to the bottom of the reservoir. These proved ineffective and were subsequently removed [28]. A hypolimnetic oxygenation system was installed in June of 2013, and operated intermittently from July to September. The system was not operated in 2014, operated intermittently in 2015, and operated continuously during periods of thermal stratification occurring in the reporting period of 2016-2017.

Almaden Lake Almaden Lake is equipped with four solar-powered hypolimnetic circulators. The first was installed at Site 1 in 2006. A second device was installed in March of 2007 (Site 2), and the remaining two were installed in January of 2009. These devices are situated in the deepest portions of the lake, which were the main pits of the historic gravel quarry. Monitoring Site 1 is used to evaluate effectiveness because it is located in primary quarry pit, and contains the most historical data. The solar circulator at Site 1 was lowered in 2007, after it was initially found to be ineffective due to its position high above the lake bottom. The effects of the lowering appeared to take effect in 2009. Due to inconsistent operation, data collected before 2009 were considered to be pre-treatment.

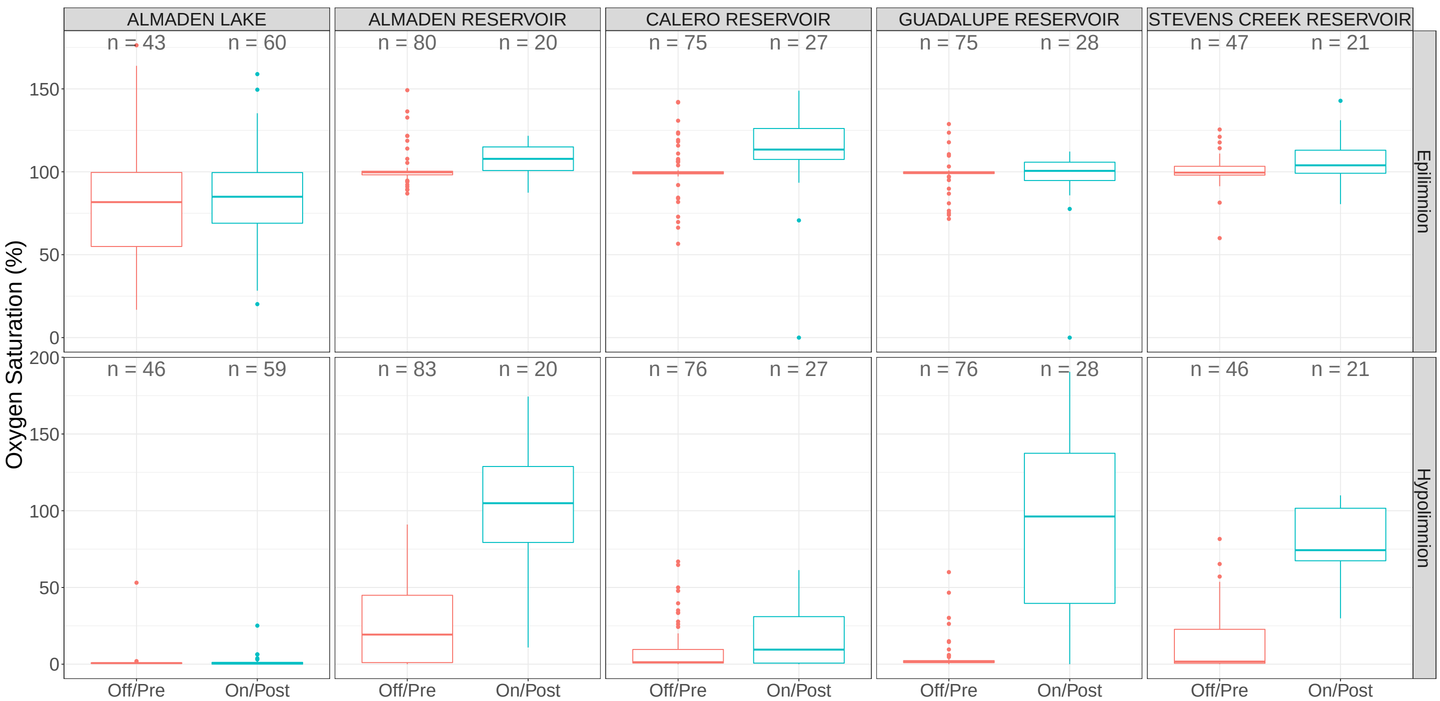
Stevens Creek Reservoir (Reference Site) A hypolimnetic oxygenation system was installed in Stevens Creek Reservoir in 2013. It operated intermittently in 2015, and continuously during periods of thermal stratification occurring in the reporting period of 2016-2017.

8.3 Water Quality Results

This section discusses the effectiveness of the treatment systems in improving dissolved oxygen concentrations, reducing total methylmercury concentrations, and decreasing internal nutrient loading in the reservoirs. Effectiveness is evaluated by comparing concentrations measured before and during system operation, limited to the dry season of May to September. During this period, reservoirs stratify and hypolimnetic oxygen depletion necessitates the operation of the treatment systems.

8.3.1 Almaden Reservoir

Figure 28: Effects of Hypolimnetic Oxygenation Systems (Reservoirs) and Solar Circulator (Lake) on Dissolved Oxygen Saturation



Dissolved Oxygen Saturation Figure 28 shows epilimnetic and hypolimnetic dissolved oxygen saturation measured prior to and during operation of the treatment systems. Prior to the installation of the hypolimnetic oxygenation system in Almaden Reservoir, hypolimnetic dissolved oxygen concentrations dropped dramatically during summer stratification, commonly reaching 0 mg/L by August (Figure 4). During operation of the system, average hypolimnetic dissolved oxygen saturation increased significantly ($p<0.001$) from 25% (SD=26) to 102% (SD=44). When operated continuously, hypolimnetic anoxia was avoided.

Total Methylmercury Figure 31 shows epilimnetic and hypolimnetic total methylmercury concentrations measured prior to and during operation of the treatment systems. Prior to the operation of the hypolimnetic oxygenation system, dry season total methylmercury concentrations averaged 1.9 ng/L (SD=2.5) in Almaden Reservoir's hypolimnion, with short-lived peaks as high as 10.5 ng/L. During the operation of the system, average total methylmercury concentrations declined 62% to 0.72 ng/L (SD=1.1), and peak concentrations were reduced to 5.4 ng/L. During the continuous operation of the system that occurred over the current reporting period, peak concentrations methylmercury concentrations did not exceed the TMDL of 1.5 ng/L in the hypolimnion. However, the observed reductions in total methylmercury concentrations were not statistically significant ($p=0.13$). Total methylmercury concentrations in Almaden Reservoir's epilimnion were not influenced significantly by the operation of the hypolimnetic oxygenation system, with mean concentrations of approximately 0.8 ng/L measured prior to and during operation.

Sulfate Reduction Figure 35 shows epilimnetic and hypolimnetic sulfate concentrations measured prior to and during operation of the treatment systems. Prior to the operation of the hypolimnetic oxygenation system, dry season sulfate concentrations averaged 14.3 mg/L (SD=1.4) in Almaden Reservoir's hypolimnion. Though average sulfate concentrations increased slightly to 14.7 mg/L (SD=0.99) during the operation of the oxygenation system, this distinction was not statistically or practically significant. These results indicate that oxygenation system operation did not have a pronounced effect on microbial sulfate reduction. This may explain our inability to find a significant decrease in total methylmercury concentrations in Almaden Reservoir's hypolimnion during system operation.

Internal Nutrient Loading

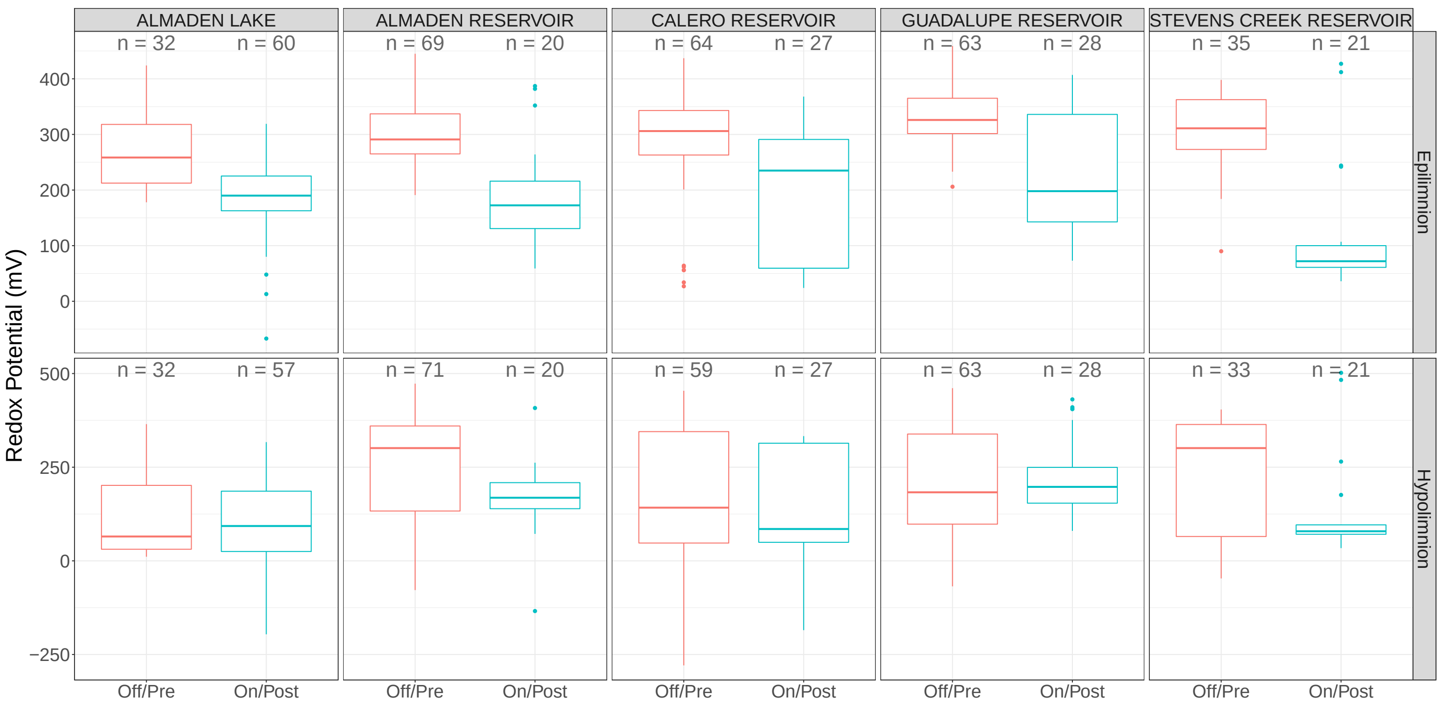
Total Phosphorus Figure 36 shows epilimnetic and hypolimnetic total phosphorus concentrations measured prior to and during operation of the treatment systems. The operation of the hypolimnetic oxygenation system in Almaden Reservoir had no significant effect on hypolimnetic total phosphorus concentrations, with average concentrations of 0.05 mg/L measured during the dry season prior to and during the operation of the system. Epilimnetic total phosphorus concentrations increased 253% from 0.016 mg/L (SD=0.03) to 0.04 mg/L (SD=0.02) during the operation of the oxygenation system, though this is not apparent in the plot due to reporting limit changes. This increase was statistically (p=0.001) and practically significant, as it elevates the reservoir from mesotrophic to eutrophic according to Carlson's Trophic State Index. Increased total phosphorus concentrations in the photic zone are likely to stimulate phytoplankton and cyanobacteria blooms.

Ammonia Figure 37 shows epilimnetic and hypolimnetic ammonia concentrations measured prior to and during operation of the treatment systems. Prior to the operation of the hypolimnetic oxygenation system in Almaden Reservoir, dry season ammonia concentrations averaged 0.21 mg/L (SD=0.27) in Almaden Reservoir's hypolimnion. During the operation of the system, average ammonia concentrations decreased 27% to 0.16 mg/L (SD=0.06). This decrease was statistically significant ($p<0.01$). There was no significant difference in epilimnetic ammonia concentrations during the operation of the hypolimnetic oxygenation system.

Chlorophyll *a* and Phycocyanin Figure 38 shows epilimnetic chlorophyll *a* and phycocyanin concentrations measured prior to and during operation of the treatment systems. Prior to the operation of the hypolimnetic oxygenation system in Almaden Reservoir, epilimnetic chlorophyll *a* concentrations averaged $3.4\,\mu g\,L^{-1}$ (SD=4) during the dry season. During the operation of the system, concentrations increased significantly ($p<0.05$) to $3.8\,\mu g\,L^{-1}$ (SD=2). Though this increase is slight, and does not change the trophic state of the reservoir, it may be caused by increased phosphorus loading to the photic zone during system operation. Epilimnetic phycocyanin concentrations were unchanged while operating the hypolimnetic oxygenation system.

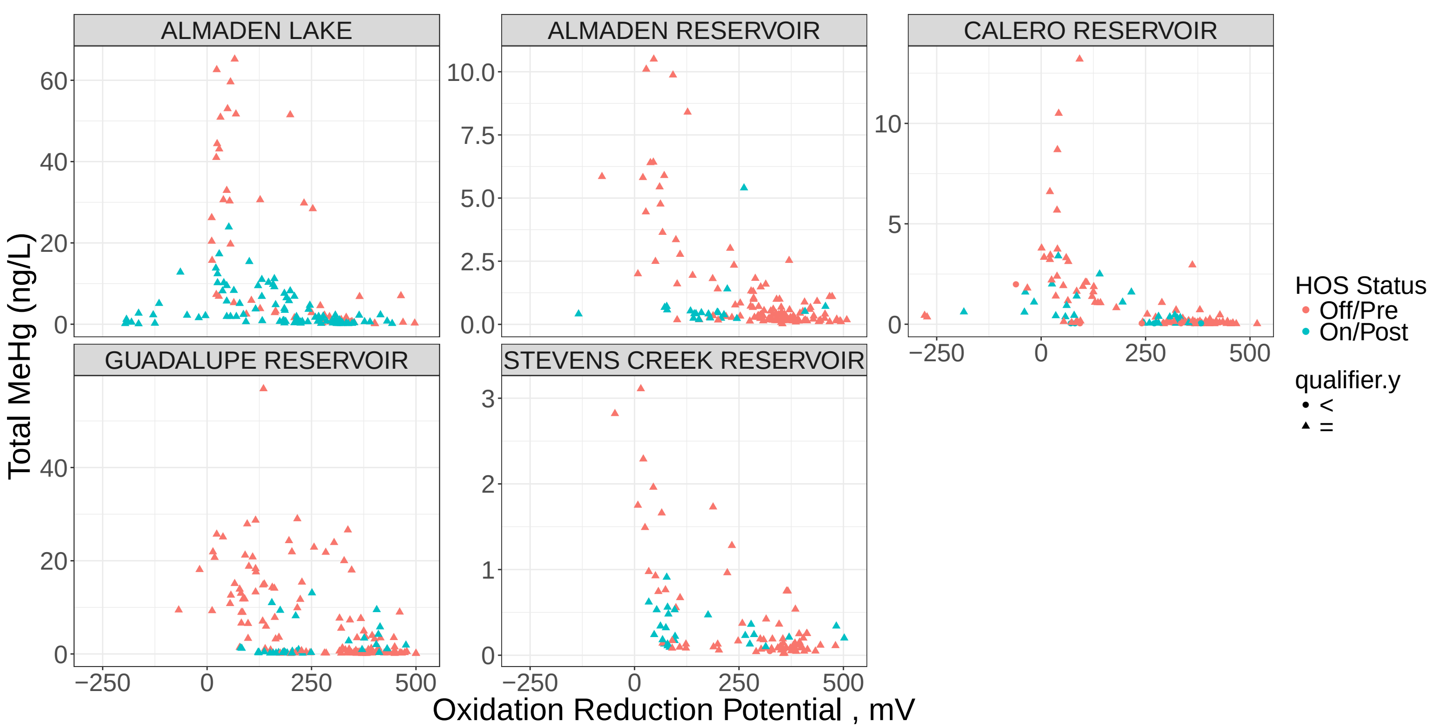
Redox Potential Figure 29 shows field-measurements of epilimnetic and hypolimnetic oxidation-reduction potential (ORP). Field measurements of ORP may be subject to high variability. ORP is temperature and pH-dependent, but not corrected for either. Additionally, ORP readings can be disturbed by air bubbles (aeration), floating particles, or biological layers [13]. Despite potential inaccuracy, ORP may be a valuable, low-cost indicator of redox changes. Negative ORP values suggest the dominance of reducing processes, which make a substance likely to lose electrons to new chemical species. After oxygen is consumed at the sediment-water interface of reservoirs, a succession of abiotic and microbially-mediated chemical reactions occur, each at a more negative ORP. Poising redox above conditions that facilitate sulfate reduction could inhibit the co-occurring methylation

Figure 29: Effects of Hypolimnetic Oxygenation Systems (Reservoirs) and Solar Circulator (Lake) on Redox Potential



of mercury. This is illustrated by the narrow redox "window" under which elevated methylmercury production occurs: generally between 0 and 125 mV in District reservoirs, with the exception of Guadalupe (Figure 30).

Figure 30: Relationship between ORP and Total Methylmercury in Reservoir and Lake Hypolimnia

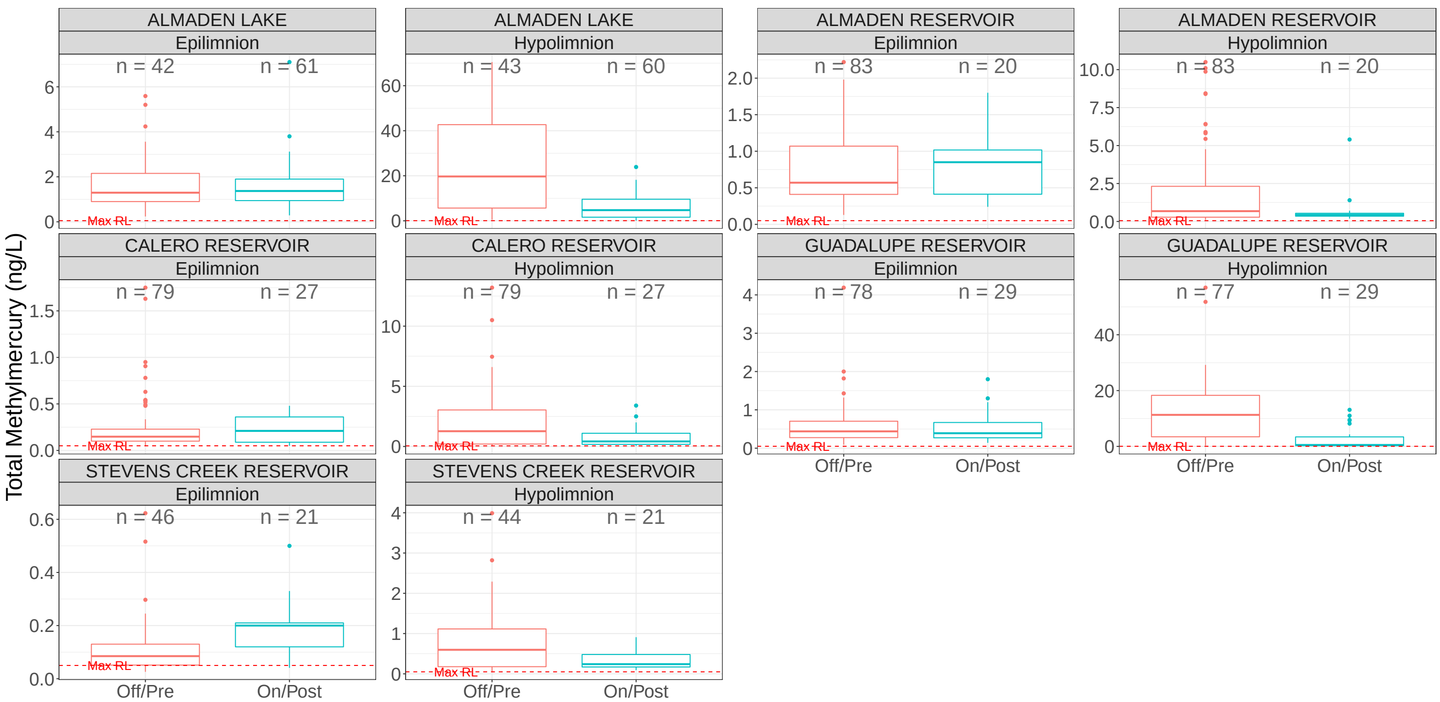


In Almaden Reservoir, dry season average hypolimnetic ORP decreased from 262 mV (SD=138) to 164 mV (SD=103) during oxygenation system operation. This decrease was statistically significant ($p<0.01$). In the epilimnion, dry season average ORP decreased significantly ($p<0.001$) from 302 mV (SD=57) to 188 mV (SD=94). This may be caused by advective transport of reducing bottom water into the epilimnion with the oxygenation system's rising bubble plumes. This, in turn, could transport profundal compounds (including Methymercury and nutrients) into the photic zone where they may be absorbed by phytoplankton, entering the aquatic food web.

8.3.2 Calero Reservoir

Dissolved Oxygen Saturation Prior to the installation of the hypolimnetic oxygenation system in Calero Reservoir, hypolimnetic dissolved oxygen concentrations dropped dramatically during summer stratification, commonly reaching 0 mg/L by July and remaining anoxic until fall turnover (Figure 4). During operation of the system, average hypolimnetic dissolved oxygen saturation increased from 8% (SD=15) to 17% (SD=19)(Figure 28). This increase was not statistically significant. Average epilimnetic dissolved oxygen saturation increased significantly ($p<0.001$) from 100% (SD=13) to 112% (SD=28). Unique of the reservoirs studied, Calero Reservoir did not efficiently retain added oxygen in its hypolimnion. Even when continuous oxygenation began prior to hypolimnetic oxygen depletion, the reservoir commonly experienced sustained anoxia during operation of the system.

Figure 31: Effects of Hypolimnetic Oxygenation Systems (Reservoirs) and Solar Circulator (Lake) on Total Methylmercury Concentration



Total Methylmercury Prior to the operation of the hypolimnetic oxygenation system, dry season total methylmercury concentrations averaged 2 ng/L (SD=2.45) in Calero Reservoir's hypolimnion, with short-lived peaks as high as 13.2 ng/L. During the operation of the system, average total methylmercury concentrations declined 60% to 0.82 ng/L (SD=0.85), and peak concentrations were reduced to 3.4 ng/L (Figure 31). This reduction in methylmercury concentration was statistically significant ($p<0.05$). During the current reporting period, total methylmercury concentrations exceeded the TMDL of 1.5 ng/L on five occasions. All exceedances occurred in the hypolimnion, and three occurred during continuous operation of the oxygenation system. Total methylmercury concentrations in Calero Reservoir's epilimnion were not influenced significantly by the operation of the hypolimnetic oxygenation system, with mean concentrations of approximately 0.25 ng/L measured prior to and during operation.

Sulfate Reduction Prior to the operation of the hypolimnetic oxygenation system, dry season sulfate concentrations averaged 23.5 mg/L (SD=7.2) in Calero Reservoir's hypolimnion. During operation of the hypolimnetic oxygenation system, average sulfate concentrations increased 32% to 31.2 mg/L (SD=7.1)(Figure 35). This difference was statistically significant ($p<0.001$). These results indicate that oxygenation system operation had a pronounced effect on microbial sulfate reduction, which was likely the mechanism by which methylmercury concentrations were reduced. Though oxygen saturation did not increase significantly, the effects of the system were sufficient to promote some suppression of sulfate reduction and methylmercury production. Epilimnetic sulfate concentrations also increased significantly ($p<0.001$), likely a result of decreased sulfate reduction in the water column or water exchange between surface and bottom waters, where sulfate concentrations decreased.

Internal Nutrient Loading

Total Phosphorus The operation of the hypolimnetic oxygenation system in Calero Reservoir had no significant effect on hypolimnetic total phosphorus concentrations, with average concentrations of approximately 0.11 mg/L measured during the dry season prior to and during the operation of the system (Figure 36). Epilimnetic total phosphorus concentrations increased 75% from 0.034 mg/L (SD=0.05) to 0.06 mg/L (SD=0.02) during the operation of the oxygenation system. This increase was statistically significant ($p=0.001$), but may not elevate the trophic class of the lake substantially. However, increased total phosphorus concentrations in the photic zone are likely to stimulate phytoplankton and cyanobacteria blooms.

Ammonia Prior to the operation of the hypolimnetic oxygenation system, dry season ammonia concentrations averaged 0.28 mg/L (SD=0.27) in Calero Reservoir's hypolimnion. During the operation of the system, average ammonia concentrations decreased 38% to 0.17 mg/L (SD=0.11)(Figure 37). This decrease was nearly statistically significant ($p=0.07$). There was no significant difference in epilimnetic ammonia concentrations during the operation of the hypolimnetic oxygenation system.

Chlorophyll *a* and Phycocyanin Prior to the operation of the hypolimnetic oxygenation system in Calero Reservoir, epilimnetic chlorophyll *a* concentrations averaged 8.2 $\mu g\, L^{-1}$ (SD=4.7) during the dry season. During the operation of the system, concentrations increased significantly ($p<0.001$) to 13.5 $\mu g\, L^{-1}$ (SD=4.9)(Figure 38). Average epilimnetic phycocyanin concentrations increased significantly ($p=0.001$) from 1,421 cells/mL (SD=865) to 3,601 cells/mL (SD=3,045) during operation of the hypolimnetic oxygenation system. Increased algal productivity may be due to increased phosphorus loading to the photic zone during system operation. These effects may be confounded by low water levels due to drought and capacity restrictions.

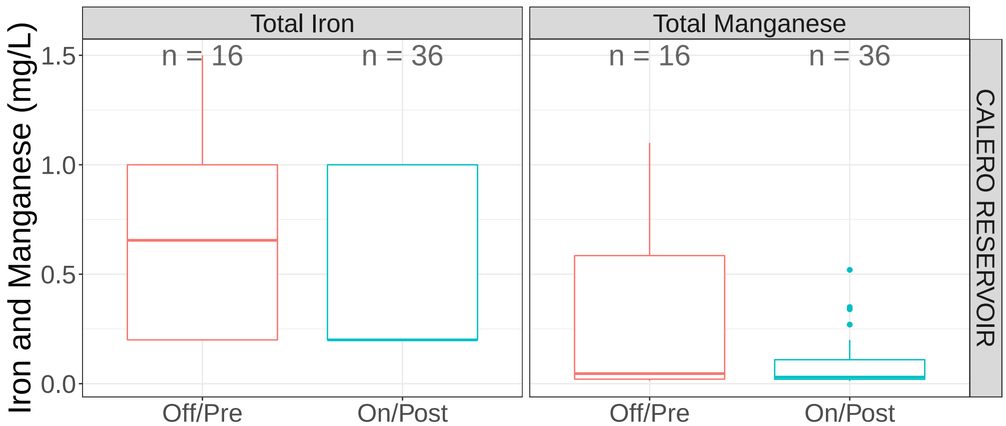
Redox Potential In Calero Reservoir, dry season average hypolimnetic ORP did not change significantly during oxygenation system operation (Figure 29). In the epilimnion, dry season average ORP decreased significantly ($p<0.001$) from 290 mV (SD=95) to 176 mV (SD=121). This may be caused by advective transport of reducing bottom water into the epilimnion with the oxygenation system's rising bubble plumes.

Redox-Sensitive Metals Total manganese and iron samples were collected from Calero Reservoir with the intention of assessing redox changes occurring during hypolimnetic oxygenation system operation. In aerobic sediments, manganese and iron occur as insoluble, oxidized forms. However, under anoxic conditions, oxidized iron and manganese are abiotically and biotically reduced to soluble forms. Dissolved manganese and iron efflux from bottom sediments increases their concentrations in the overlying water column. Thus, maintenance of aerobic conditions at the sediment-water interface should increase redox potential and reduce concentrations of manganese and iron in the hypolimnion.

Though limited historical data exists, the hypolimnetic oxygenation system at Calero Reservoir appears to have altered the sediment redox potential toward more oxidizing conditions during system operation. During operation of the system, hypolimnetic total manganese concentrations decreased significantly ($p<0.05$) from 0.48

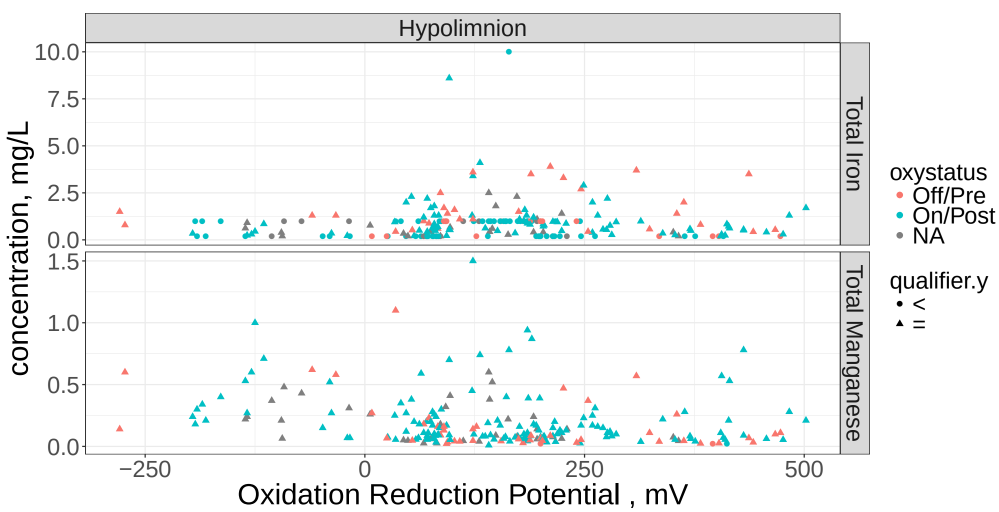
mg/L (SD=0.37) to 0.16 mg/L (SD=0.13), and total iron concentrations decreased significantly ($p<0.001$) from 0.84 mg/L (SD=0.47) to 0.39 mg/L (SD=0.22) (Figure 32).

Figure 32: Effects of Calero Reservoir Hypolimnetic Oxygenation Systems on Total Manganese and Iron Concentrations



Despite these findings, redox potential and dissolved oxygen concentration are poor predictors of total manganese and iron concentrations in hypolimnetic water (Figures 33 and 34). For this reason, these analytes will not be considered further to assess treatment system effectiveness.

Figure 33: Relationship between Measured Redox Potential and Redox-Sensitive Metals

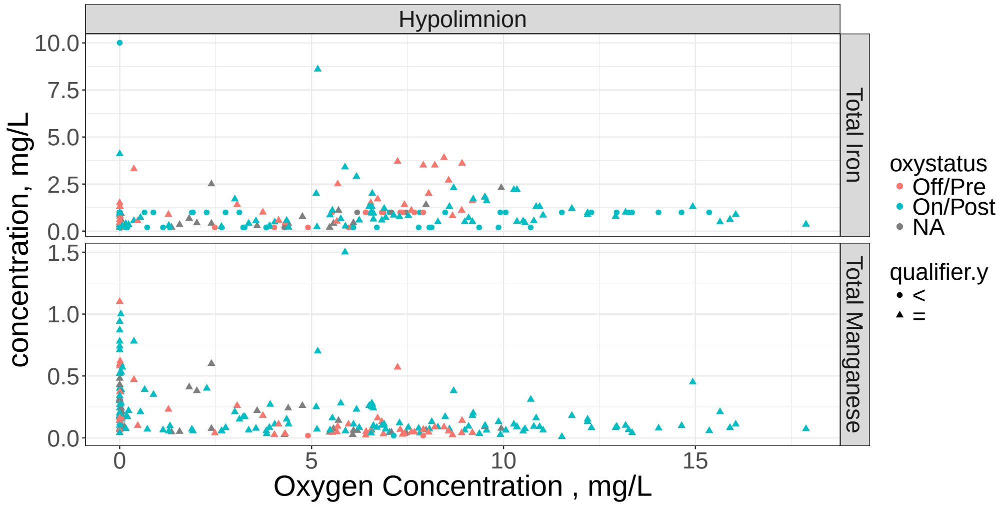


8.3.3 Guadalupe Reservoir

Dissolved Oxygen Saturation Prior to the installation of the hypolimnetic oxygenation system in Guadalupe Reservoir, hypolimnetic dissolved oxygen concentrations dropped dramatically during summer stratification, commonly reaching 0 mg/L by June and remaining anoxic until October (Figure 4). During operation of the system, average hypolimnetic dissolved oxygen saturation increased significantly ($p<0.001$) from 4% (SD=10) to 92% (SD=64)(Figure 28). During continuous operation of the system, hypolimnetic anoxia was avoided. Guadalupe Reservoir's large depth to surface area ratio and small, stable hypolimnion may enhance oxygen retention.

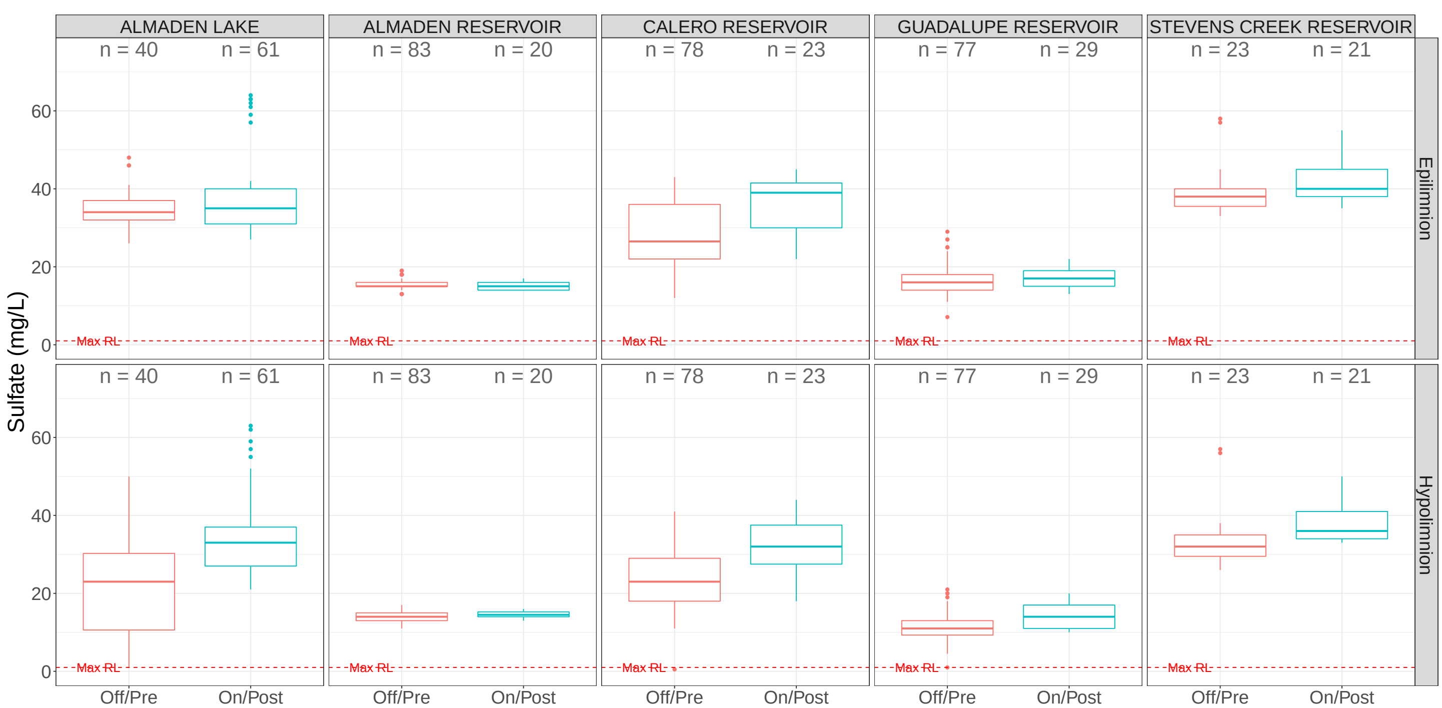
Total Methylmercury Prior to the operation of the hypolimnetic oxygenation system, dry season total methylmercury concentrations averaged 12.3 ng/L (SD=11.1) in Guadalupe Reservoir's hypolimnion, with short-

Figure 34: Relationship between Dissolved Oxygen Concentration and Redox-Sensitive Metals



lived peaks as high as 56.9 ng/L. During the operation of the system, average total methylmercury concentrations declined 80% to 2.6 ng/L (SD=3.8), and peak concentrations were reduced to 13.1 ng/L (Figure 31). This reduction in methylmercury concentration was statistically significant ($p<0.001$). During the continuous operation of the system that occurred over the current reporting period, total methylmercury concentrations did not exceed the TMDL of 1.5 ng/L. Total methylmercury concentrations in Guadalupe Reservoir's epilimnion were not statistically different during the operation of the hypolimnetic oxygenation system when compared to pre-treatment conditions.

Figure 35: Effects of Hypolimnetic Oxygenation Systems (Reservoirs) and Solar Circulator (Lake) on Sulfate Concentrations



Sulfate Reduction Prior to the operation of the hypolimnetic oxygenation system, dry season sulfate concentrations averaged 11.7 mg/L (SD=3.6) in Guadalupe Reservoir's hypolimnion. During operation of the hypolimnetic oxygenation system, average sulfate concentrations increased 20% to 14.1 mg/L (SD=2.9)(Figure

35). This difference was statistically significant ($p<0.001$). This indicates that oxygenation system operation reduced microbial sulfate reduction, which was likely the mechanism by which methylmercury concentrations were reduced. Epilimnetic sulfate concentrations were unchanged.

Internal Nutrient Loading

Total Phosphorus Prior to the operation of the oxygenation system in Guadalupe Reservoir, dry season hypolimnetic total phosphorus concentrations averaged 0.08 mg/L (SD=0.07). During operation of the hypolimnetic oxygenation system, total phosphorus concentrations were reduced 40% to 0.05 mg/L (SD=0.03)(Figure 36). This reduction was statistically significant ($p<0.05$). Epilimnetic total phosphorus concentrations increased 60% from 0.021 mg/L (SD=0.08) to 0.033 mg/L (SD=0.01) during the operation of the oxygenation system. This increase was statistically significant ($p=0.001$). Increased total phosphorus concentrations in the photic zone could stimulate phytoplankton and cyanobacteria blooms during the dry season.

Ammonia Prior to the operation of the hypolimnetic oxygenation system, dry season ammonia concentrations averaged 0.45 mg/L (SD=0.41) in Guadalupe Reservoir's hypolimnion. During the operation of the system, average ammonia concentrations decreased 69% to 0.14 mg/L (SD=0.1)(Figure 37). This decrease was statistically significant ($p<0.001$). Epilimnetic ammonia concentrations decreased 6% from 0.13 mg/L (SD=0.08) to 0.12 mg/L (SD=0.04). While this reduction was statistically significant ($p<0.01$), it may not have an appreciable effect on biological productivity.

Chlorophyll *a* and Phycocyanin Epilimnetic chlorophyll *a* concentrations were not affected by operation of the hypolimnetic oxygenation system (Figure 38). Though average epilimnetic phycocyanin concentrations increased from 260 cells/mL (SD=174) to 503 cells/mL (SD=581) during the operation of the system, median concentrations remained similar. The difference in phycocyanin concentrations measured before and during system operation was not statistically significant. While increased phosphorus loading to the epilimnion during operation of the hypolimnetic oxygenation system could stimulate algal productivity, this effect may be mitigated by reduced ammonia concentrations.

Redox Potential In Guadalupe Reservoir, dry season average hypolimnetic ORP did not change significantly during oxygenation system operation (Figure 29). In the epilimnion, dry season average ORP decreased significantly ($p<0.001$) from 331 mV (SD=51) to 228 mV (SD=107). This may be caused by advective transport of reducing bottom water into the epilimnion with the oxygenation system's rising bubble plumes.

8.3.4 Almaden Lake

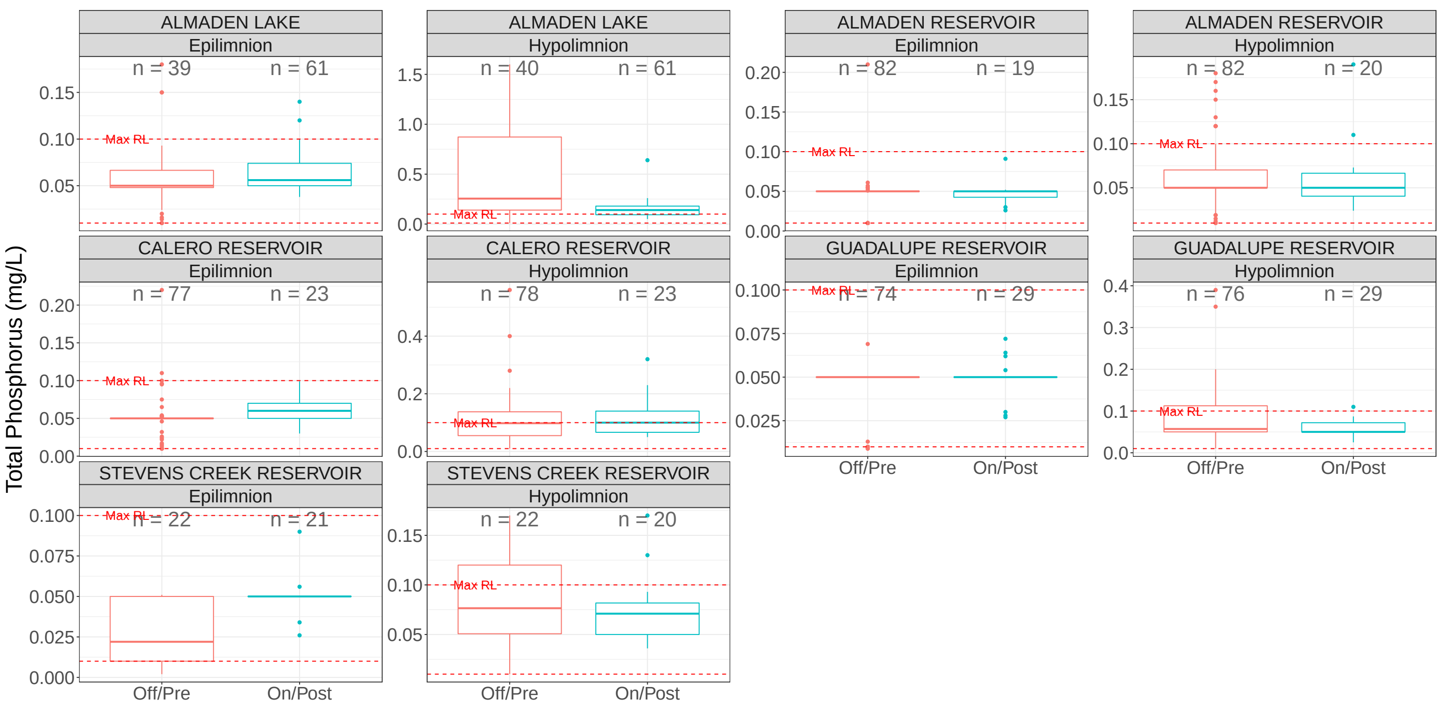
Dissolved Oxygen Saturation Prior to the installation of the solar circulator at Site 1 of Almaden Lake, hypolimnetic dissolved oxygen concentrations dropped dramatically during summer stratification, and the lake typically remained anoxic from April through September (Figure 4). The solar circulator had no significant effect on dissolved oxygen saturation in the hypolimnion or epilimnion (Figure 28).

Total Methylmercury Prior to the installation of the solar circulator at Site 1 of Almaden Lake, hypolimnetic total methylmercury concentrations averaged 25.5 ng/L (SD=21.6) during the dry season, with short-lived peaks as high as 70.5 ng/L. Following the installation of the circulator, average hypolimnetic total methylmercury concentrations declined 76% to 6 ng/L (SD=5.4), and peak concentrations were reduced to 23.9 ng/L (Figure 31). This reduction in methylmercury concentration was statistically significant ($p<0.001$). Epilimnetic total methylmercury concentrations were unchanged following the installation of the solar circulator. Though the solar circulators failed to significantly increase dissolved oxygen concentrations at Site 1, it is possible that redox conditions were enhanced sufficiently to delay the onset of reducing processes and attenuate sulfate reduction. Dilution due to mechanical dispersion of profundal compounds throughout the water column is also likely to play a role. During the current reporting period, 37% of all samples exceeded the TMDL of 1.5 ng/L.

Sulfate Reduction Prior to the installation of the solar circulator at Site 1 of Almaden Lake, dry season sulfate concentrations averaged 21.2 mg/L (SD=12.9) in the hypolimnion. Following the installation of the circulator, average sulfate concentrations increased 61% to 34.1 mg/L (SD=9.7)(Figure 35). This difference was statistically significant ($p<0.001$). Average epilimnetic sulfate concentrations were unchanged following the installation of the circulator. Though modest oxygen additions due to circulation likely delay sulfate reduction, a portion of the increased hypolimnetic sulfate concentrations may be a result of mixing sulfate-rich epilimnetic waters throughout the water column. This could provide an excess of sulfate available for microbial metabolism, reducing methylmercury concentrations by sulfide inhibition.

Internal Nutrient Loading

Figure 36: Effects of Hypolimnetic Oxygenation Systems (Reservoirs) and Solar Circulator (Lake) on Total Phosphorus Concentrations



Total Phosphorus Following the installation of the solar circulator at Site 1 of Almaden Lake, average hypolimnetic total phosphorus concentrations were reduced by 70% from 0.5 mg/L (SD=0.43) to 0.15 mg/L (SD=0.09)(Figure 36). This reduction was statistically significant ($p<0.001$). Average epilimnetic total phosphorus concentrations increased by 20% to 0.06 mg/L (SD=0.02), but this difference was not statistically significant ($p=0.09$).

Ammonia Following the installation of the solar circulator at Site 1 of Almaden Lake, average hypolimnetic ammonia concentrations were reduced by 73% from 2.33 mg/L (SD=2.3) to 0.62 mg/L (SD=0.45)(Figure 37). This reduction was statistically significant ($p<0.001$), indicating oxidation of ammonia with improved redox conditions. Epilimnetic ammonia concentrations were unchanged.

Chlorophyll *a* and Phycocyanin Epilimnetic chlorophyll *a* concentrations increased significantly following the installation of the solar circulator at Site 1 of Almaden Lake ($p<0.05$). Following the installation, average chlorophyll *a* concentrations increased 72% from $13.7\,\mu g\,L^{-1}$ (SD=13) to $23.6\,\mu g\,L^{-1}$ (SD=21.1)(Figure 38). This may be a result of the circulator mechanically mobilizing profundal nutrients into the photic zone where algae growth occurs. Phycocyanin concentrations were not significantly affected by the installation of the solar circular.

Redox Potential Dry season average hypolimnetic ORP did not change significantly following the installation of the solar circulator in Site 1 of Almaden Lake (Figure 29). In the epilimnion, dry season average ORP decreased significantly ($p<0.001$) from 275 mV (SD=70) to 186 mV (SD=66). This may be caused by the circulator mechanical dispersing reducing bottom water into the epilimnion.

8.3.5 Reference Site: Stevens Creek Reservoir

Dissolved Oxygen Saturation Prior to the installation of the hypolimnetic oxygenation system in Stevens Creek Reservoir, hypolimnetic dissolved oxygen concentrations dropped dramatically during summer stratification, commonly reaching 0 mg/L by July and remaining anoxic until September (Figure 4). During operation of the system, average hypolimnetic dissolved oxygen saturation increased significantly ($p<0.001$) from 14% (SD=21) to 83% (SD=23)(Figure 28). During continuous operation of the system, hypolimnetic anoxia was avoided, but the thermocline occasionally became hypoxic late in the summer.

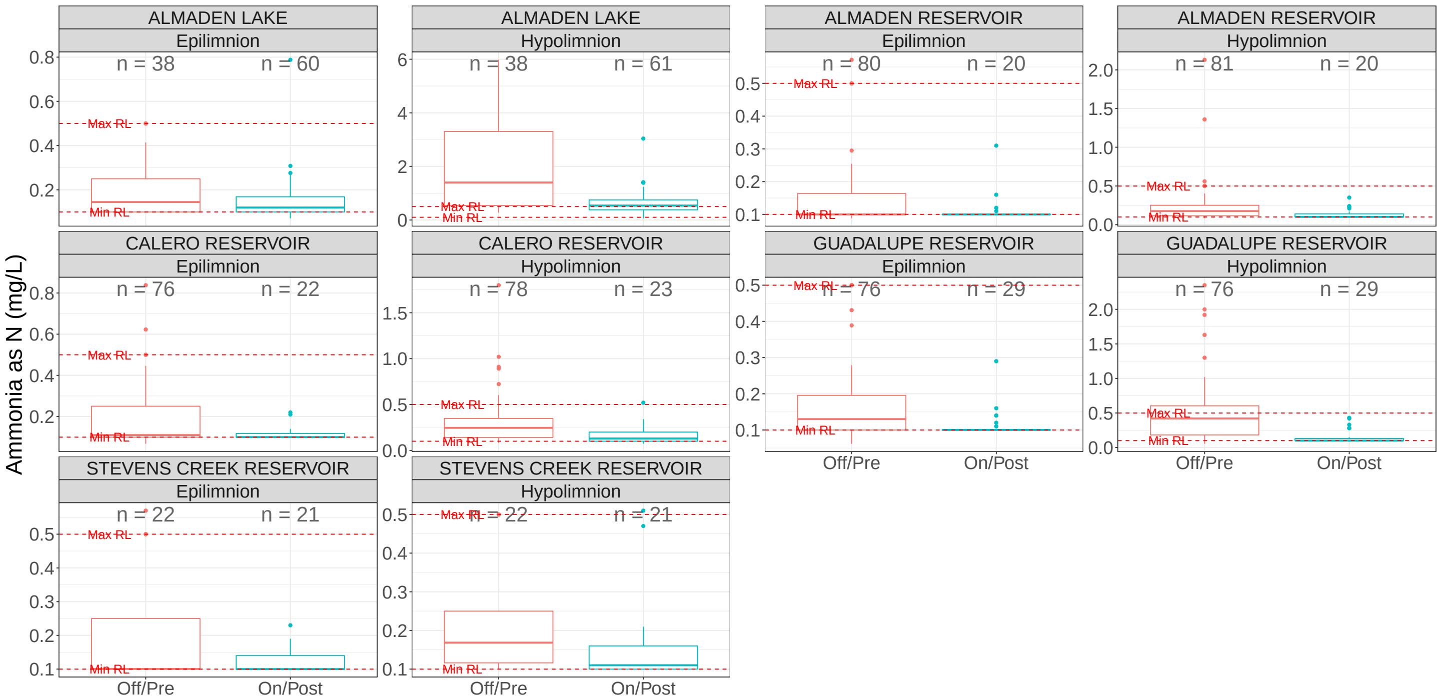
Total Methylmercury Prior to the operation of the hypolimnetic oxygenation system, dry season total methylmercury concentrations averaged 0.83 ng/L (SD=0.85) in Stevens Creek Reservoir's hypolimnion, with short-lived peaks as high as 4 ng/L. During the operation of the system, average total methylmercury concentrations declined 60% to 0.33 ng/L (SD=0.21), and peak concentrations were reduced to 0.91 ng/L (Figure 31). This reduction in methylmercury concentration was statistically significant ($p<0.05$). During the continuous operation of the system that occurred over the current reporting period, total methylmercury concentrations did not exceed the TMDL (for water bodies of the Guadalupe River Watershed) of 1.5 ng/L in the hypolimnion. However, concentrations exceeded 1.5 ng/L on 4 occasions in the thermocline during low-oxygen conditions. Average epilimnetic total methylmercury concentrations increased 62% from 0.12 ng/L (SD=0.11) to 0.19 ng/L (SD=0.11) during the operation of the hypolimnetic oxygenation system. This increase was statistically significant ($p<0.001$). Since methylmercury enters the food web through passive diffusion into phytoplankton, which predominantly occupy the photic zone, this increase could accelerate bioaccumulation during the dry season.

Sulfate Reduction Prior to the operation of the hypolimnetic oxygenation system, dry season sulfate concentrations averaged 33.7 mg/L (SD=7.8) in Stevens Creek Reservoir's hypolimnion. During operation of the hypolimnetic oxygenation system, average sulfate concentrations increased 14% to 38.7 mg/L (SD=6.2)(Figure 35). This difference was statistically significant ($p<0.001$), and indicates decreased microbial sulfate reduction. Average epilimnetic sulfate concentrations increased from 39.3 mg/L (SD=6.4) to 42.1 mg/L (SD=6.2) during operation of the system. This difference was nearly significant ($p=0.07$).

Internal Nutrient Loading

Total Phosphorus Though average hypolimnetic total phosphorus concentrations decreased by 50% during the operation of the oxygenation system in Stevens Creek Reservoir, median concentrations were similar before and during operation (Figure 36). There was no statistically significant difference in hypolimnetic total phosphorus concentrations measured before and during system operation. However, average epilimnetic total phosphorus concentrations increased 380% from 0.009 mg/L (SD=0.015) to 0.035 mg/L (SD=0.02) while the system was operated. This increase was statistically significant ($p<0.05$), and could stimulate primary productivity in the photic zone.

Figure 37: Effects of Hypolimnetic Oxygenation Systems (Reservoirs) and Solar Circulator (Lake) on Ammonia Concentrations



Ammonia The hypolimnetic oxygenation system ostensibly reduced epilimnetic and hypolimnetic ammonia concentrations in Stevens Creek Reservoir (Figure 37), but these effects were not statistically significant. The reservoir's low baseline concentrations, yielding many non-detect values, decrease our ability to detect a reduction in ammonia during oxygenation.

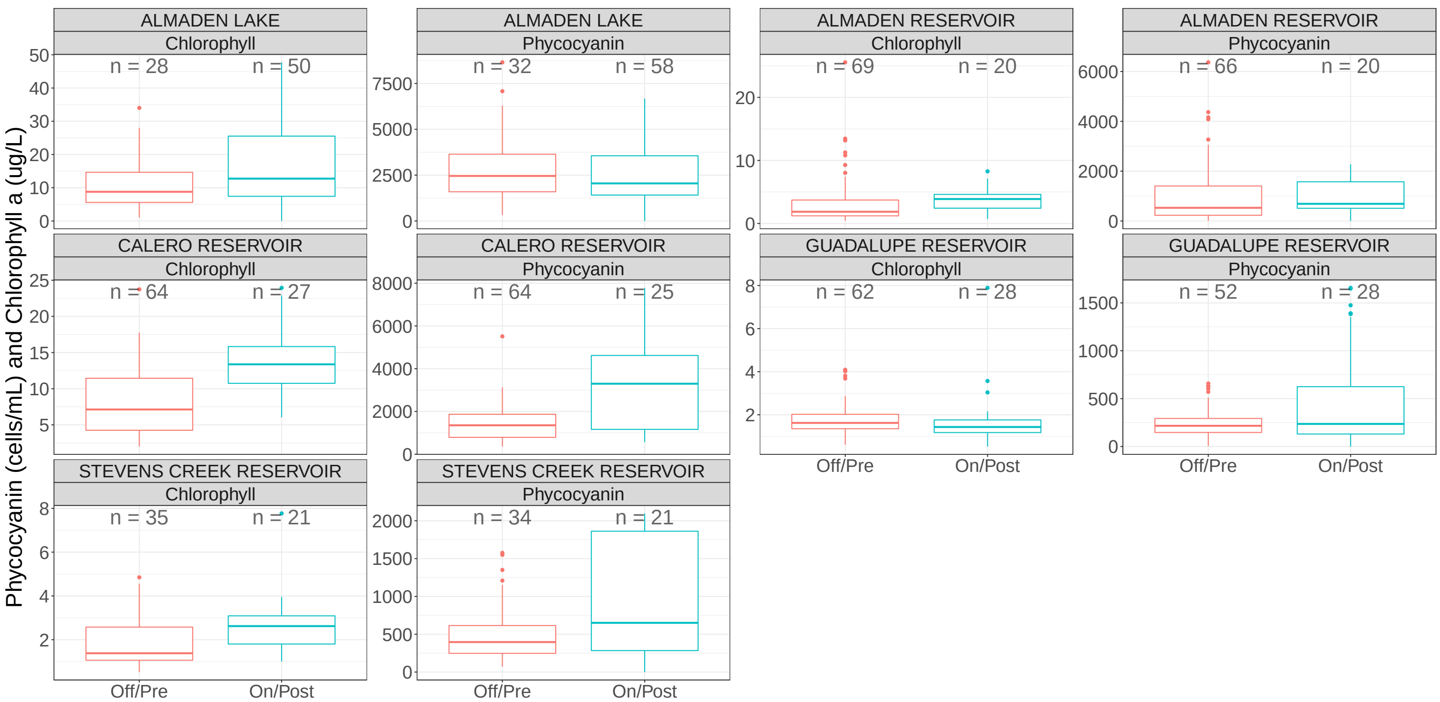
Chlorophyll *a* and Phycocyanin Epilimnetic chlorophyll *a* concentrations increased significantly during the operation of the hypolimnetic oxygenation system in Stevens Creek Reservoir ($p<0.05$). While operating the system, average chlorophyll *a* concentrations increased 42% from 1.9 $\mu g\,L^{-1}$ (SD=1.3) to 2.7 $\mu g\,L^{-1}$ (SD=1.4) (Figure 38). Though average epilimnetic phycocyanin concentrations increased from 520 to 900 cells/mL, this discrepancy was not statistically significant.

Redox Potential Dry season average hypolimnetic ORP decreased from 224 mV (SD=152) to 127 mV (SD=130) following the installation of the hypolimnetic oxygenation system in Stevens Creek Reservoir (Figure 29), but this difference was not statistically significant ($p=0.9$). In the epilimnion, dry season average ORP decreased significantly ($p<0.001$) from 305 mV (SD=72) to 120 mV (SD=113). This may be caused by advective transport of reducing bottom water into the epilimnion with the oxygenation system's rising bubble plumes.

8.3.6 Oxygenation System Performance

The hypolimnetic oxygenation systems are designed to deliver 0.75 US tons of oxygen per day. In the initial study used to size the systems, Reed and Graham used dissolved oxygen profile data collected from 1999 to 2002 to calculate aerobic oxygen demands of Almaden, Guadalupe, and Calero Reservoirs. Oxygen demands were

Figure 38: Effects of Hypolimnetic Oxygenation Systems (Reservoirs) and Solar Circulator (Lake) on Chlorophyll a and Phycocyanin Concentrations

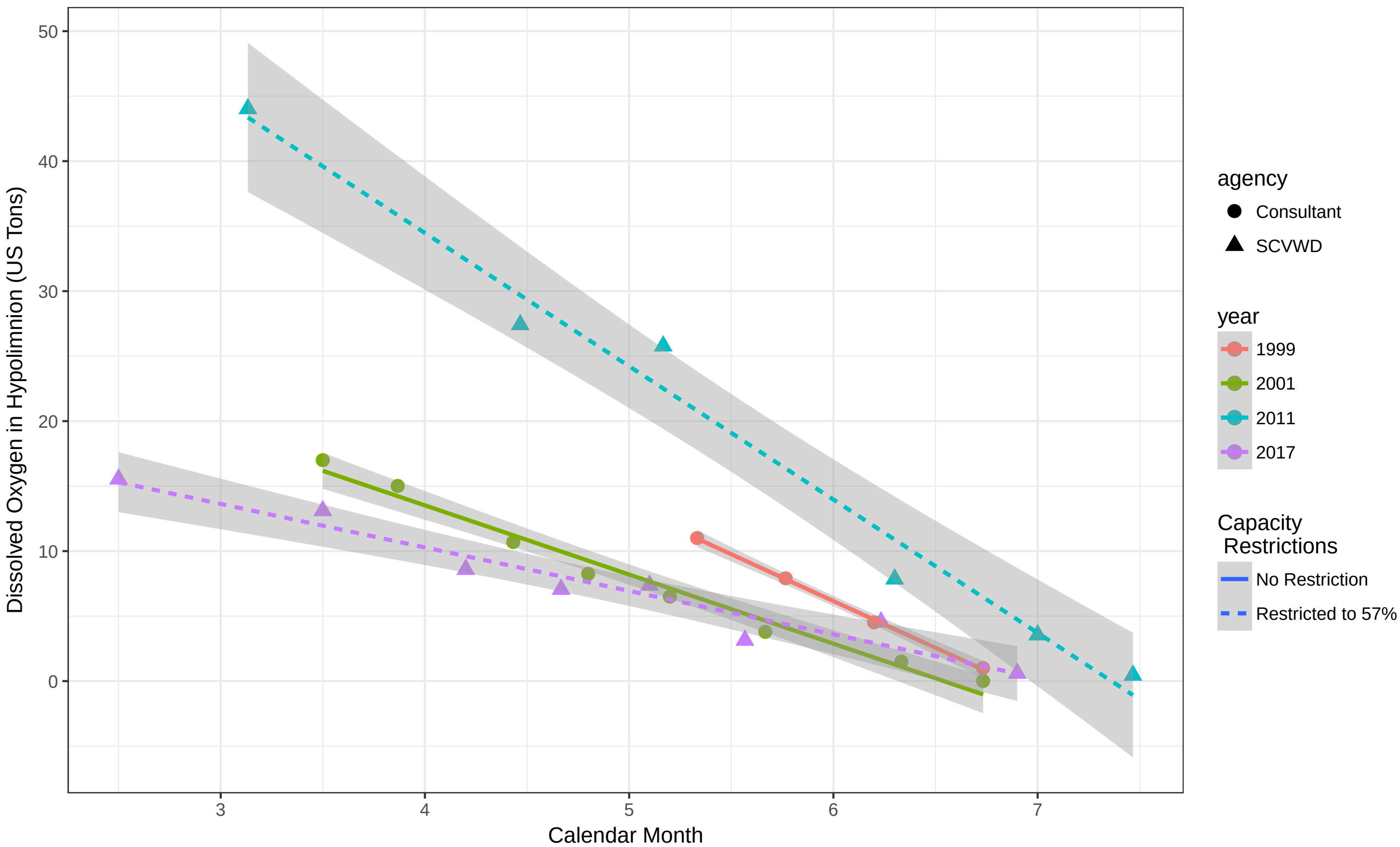


calculated with 99.9% confidence as 0.15 US tons/day, 0.26 US tons/day, and 0.34 US tons/day, respectively [65]. During the current reporting period, the hypolimnetic oxygenation systems were operated nearly-continuously during the stratification period, apart from brief shut-downs due to mechanical failure. In 2017, the system at Calero Reservoir was nonoperational until June 19, after its hypolimnion became anoxic. During system operation, dissolved oxygen concentrations increased significantly in the hypolimnia of all reservoirs except Calero. To investigate if oxygen demand has increased since 2002, rendering Calero Reservoir's hypolimnetic oxygenation system undersized, District staff calculated current oxygen demand rates using data collected in 2011 (before the system was installed) and 2017 (while the system was nonoperational). Reservoir capacity restrictions were imposed in 2010, potentially affecting oxygen demand. The results indicate the 2011 oxygen demand as 0.23 US tons/day and the 2017 oxygen demand as 0.11 US tons/day (Figure 39). We therefore conclude that the system is properly sized to meet the aerobic oxygen demand of the reservoir, and that some other mechanism is responsible for the absence of increased oxygen concentrations.

Lake sediments commonly have an "oxygen debt" evident by non-biological uptake of oxygen by reduced substances formed under anaerobic conditions [66]. Under anoxic conditions, nitrification of ammonium is curtailed, leaving it to accumulate in bottom-waters. When oxygen is again introduced to the sediments (by turnover or hypolimnetic oxygenation), much of it may be used to oxidize the accumulated ammonium through nitrification, decreasing oxygen available to aerobic bacteria. With sufficient buildup of reduced chemical species, the oxygen debt required for their oxidation may exceed the oxygen demand of aerobic bacteria. Thus, if anoxic conditions are established, subsequent oxygen demand may increase to the point that the current system capacity is insufficient to keep pace with non-biological oxygen uptake. This would inhibit the accumulation of oxygen in the hypolimnion. Although dissolved oxygen concentrations did not increase significantly in Calero Reservoir, the added oxygen likely increased the redox state sufficiently to curtail sulfate reduction. Because some iron-reducing bacteria are known to methylate mercury, effective controls would avoid anoxic conditions at the sediment-water interface.

Though smaller reservoirs can be brought from anoxic conditions to full oxygen saturation relatively quickly, this may be impossible at Calero Reservoir due to its large volume and high sediment surface area. Duvil et al. calculated an anaerobic ammonia flux rate of 16.6 mg-N/m^2/day in Guadalupe Reservoir [32]. Given the

Figure 39: Calculated Oxygen Demands in Calero Reservoir



reservoir's surface area of seasonally-anoxic sediments (appx. 27 acres), this equates to a total NH_3 flux of 0.026 US tons per day. Because only 0.03 US tons of oxygen per day are needed to oxidize this mass of ammonia, the hypolimnetic oxygenation system's 0.75 US ton per day delivery capacity is more than sufficient to oxidize NH_3 and other reduced substances accumulated during anoxia, as well as amass excess oxygen. Though Calero Reservoir is considerably more eutrophic than Guadalupe Reservoir and likely experiences greater ammonia flux, applying the same flux rate to Calero Reservoir's seasonally anoxic sediment surface area (appx. 95 acres) yields a total NH_3 load of 0.1 US tons per day. Oxidation of ammonia alone would consume 0.12 tons of oxygen per day. Combine this with the abiotic oxidation of other reduced species accumulated under anoxic conditions (Fe, Mn, HgS), and anaerobic oxygen demand could outweigh the system's oxygen delivery capacity. Empirically-measured anaerobic flux rates of reduced species may be necessary to estimate abiotic oxygen demand during anoxic conditions. To avoid building an oxygen debt, it is imperative that the operation of Calero Reservoir's hypolimnetic oxygenation system begins well before the sediment-water interface becomes hypoxic.

Alternatively, Calero Reservoir's unique thermal stratification regime may prevent efficient oxygen retention. Calero Reservoir has a large surface area relative to its mean depth, elevating surface temperatures and facilitating wind-driven mixing. Whereas the other reservoirs studied support stable thermal stratification with epilimnia and hypolimnia of distinct temperatures and densities, Calero Reservoir often exhibits a more linear relationship between temperature and depth. It is possible that, without a uniform hypolimnion, added oxygen diffuses upward into the water column instead of being retained in bottom-waters. This may be why the increase in epilimnetic oxygen saturation is more pronounced in Calero Reservoir than the other reservoirs. However, this increase in epilimnetic oxygen saturation may be a result of increased algal productivity during system operation.

8.3.7 Summary: Effectiveness in Improving Water Quality

Line-Diffuser Hypolimnetic Oxygenation Systems In all reservoirs except Calero Reservoir, the hypolimnetic oxygenation systems dramatically improved dissolved oxygen concentrations in bottom waters. In reservoirs where oxygen increased, the systems were laterally effective, with high oxygen concentrations measured up to one thousand feet from the diffuser line (Appendix B.1-B.3). Added oxygen saturates the hypolimnion, but does accumulate in the thermocline because of its upwardly-increasing temperature and decreasing density. Sulfate concentrations increased significantly in the hypolimnia of all reservoirs except Almaden Reservoir. Correspondingly, all reservoirs except Almaden experienced significant reductions in hypolimnetic methylmercury concentrations during system operation. This suggests that methylmercury production was reduced by attenuating microbial sulfate reduction. However, a portion of the perceived decreases in hypolimnetic methylmercury concentration may be due to mechanical homogenization of the hypolimnia by the turbulence of the bubble plumes. In all reservoirs except Guadalupe, reduction of hypolimnetic methylmercury concentrations results in decreased loading from the reservoir, because outlet works withdrawal water from the reservoir bottoms. Despite the significant reductions in the hypolimnia of the reservoirs, methylmercury concentrations either remained constant or increased in the epilimnia during system operation. This is concerning, because methylmercury enters the food chain by passive diffusion into phytoplankton, predominantly occurring in the photic zone. Theoretically, without reducing phytoplankton methylmercury concentrations, it is unlikely that concentrations would be reduced in fish. However, chlorophyll *a* concentrations increased in all reservoirs except Guadalupe during operation of the hypolimnetic oxygenation systems. Increased algal biomass may reduce mercury concentrations in fish through bloom dilution. Though ammonia and phosphorus efflux from bottom sediments were sometimes reduced during operation of the oxygenation systems, epilimnetic phosphorus concentrations increased in all reservoirs. This may be the cause of the observed increases in chlorophyll *a* concentrations. It is possible that the increases in total methylmercury and phosphorus concentrations in the epilimnia of the reservoirs could be due to advection created by the upward motion of the oxygenation systems' bubble plumes. This is supported by decreased ORP in the epilimnia, which may be due to mixing with reducing bottom-waters introduced to the photic zone. Despite the significant findings of this study, mercury and nutrient cycling are prone to strong inter-annual variability, so some effects may be stochastic and influenced more by seasonality than system operation.

Reservoir Turnover The literature commonly describes methylmercury and nutrient pulses released to the photic zone during fall turnover. During periods of anoxia, nutrients, dissolved metals, and methylmercury accumulate in the hypolimnia of lakes and reservoirs. At turnover, profundal nutrients and other compounds are mixed throughout the water column, increasing the availability of methylmercury and nutrients to phytoplankton, which occupy the photic zone. This is thought to cause algae blooms, and the pulse of methylmercury observed in fish shortly after fall turnover [71].

Our data indicates slight increases in total phosphorus concentrations measured in the photic zone during the months of October and November, though differences are only significant in Almaden Lake. It is difficult to attribute these increases to reservoir turnover, since the "first flush" of stormwater runoff usually occurs during this period. Ammonia, which is primarily produced by internal processes, did not increase in the photic zone following reservoir turnover. Nonetheless, chlorophyll *a* and phycocyanin concentrations were notably higher in Almaden Lake, Almaden Reservoir, and Guadalupe Reservoir during the months of October and November than during the rest of the year.

On a two-week monitoring interval, we have never observed an increase in aqueous methylmercury in the photic zone following reservoir turnover. It is probably the case that phytoplankton rapidly absorb this methylmercury pulse, and we are unable to detect it on our monitoring interval. However, the effects of hypolimnetic withdrawal and discharge may decrease methylmercury concentrations available at turnover. Since reservoirs destratify from top to bottom and peak methylmercury concentrations are generally observed in August, it is plausible that, by the time the epilimnia cool sufficiently to achieve turnover, much of the hypolimnetic methylmercury pool has been discharged downstream (Figure 40). Thus, dry season epilimnetic methylmercury concentrations are likely an important factor controlling methylmercury concentrations in the aquatic food web. Hypolimnetic entrainment is likely to occur throughout the dry season, as the thermocline slowly descends during the summer months. This could cause a slow diffusion of methylmercury into surface waters as stratification evolves, before the pulse that may occur at turnover (Figure 41).

Figure 40: Hypolimnetic Methylmercury Concentrations at Fall Turnover

Blue rectangles reflect ranges of turnover timing, and red numbers reflect sample sizes.

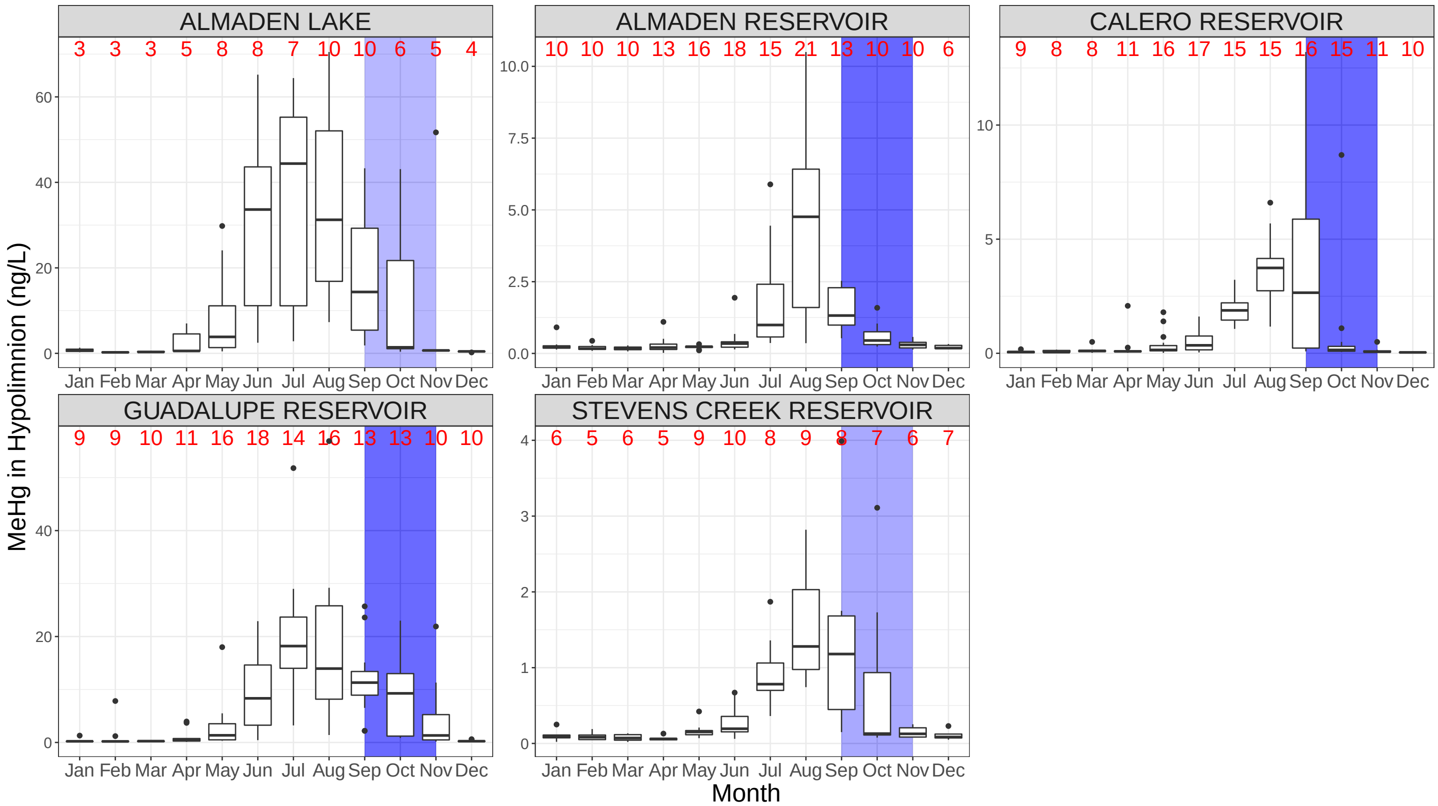
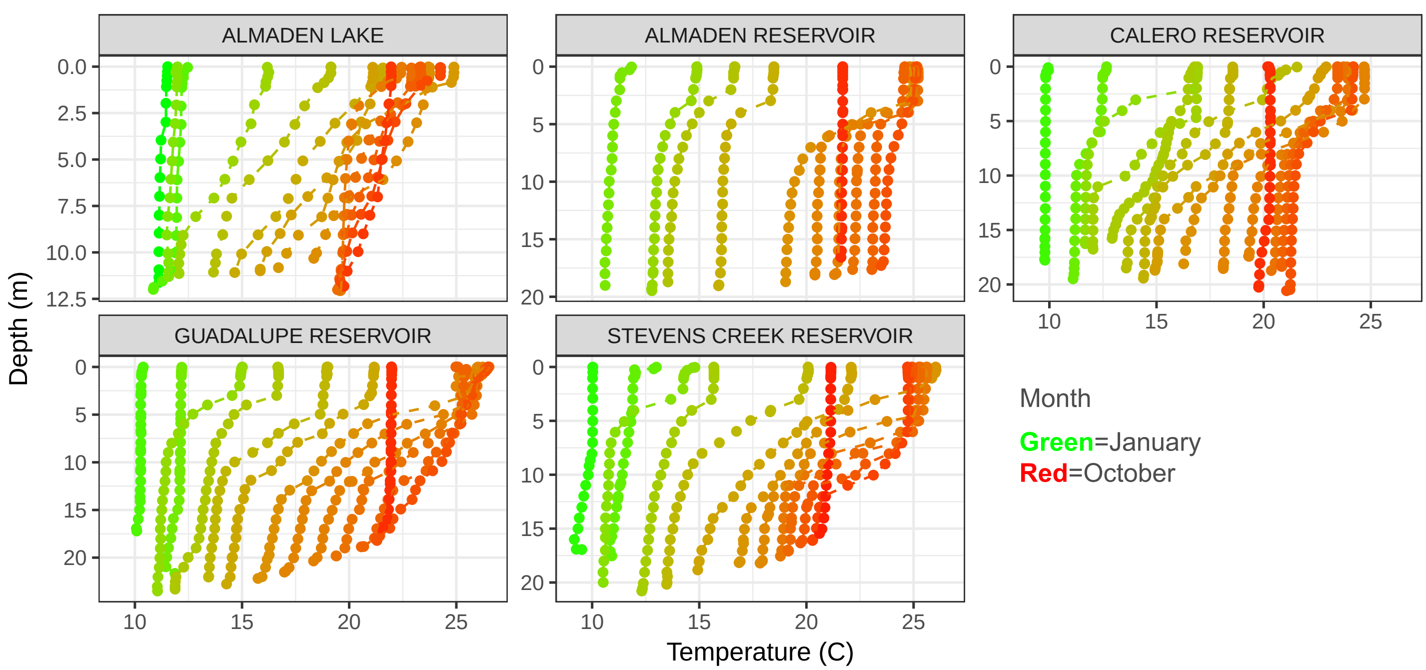


Figure 41: Typical Temperature Profiles of Reservoirs and Lake (2017)



Seasonal variations in fish foraging patterns are likely to affect their concentration of methylmercury. When water temperatures cool in the wet season, sunfish metabolism slows as feeding declines [82]. Since rapid feeding and growth occur primarily during the summer months, aquatic chemistry during the dry season is likely more important in controlling mercury uptake than that occurring following reservoir turnover when feeding slows.

To test the effects of the oxygenation systems on decreasing nutrient and methylmercury concentrations introduced to the photic zone during fall turnover, we compared water quality results measured in October and November of years when the oxygenation systems were and were not operated. Though the post-operation sample size is small, we were unable to detect decreases in total methylmercury, total phosphorus, ammonia, chlorophyll *a*, or phycocyanin concentrations in the months of October and November following seasons of oxygenation. Almaden Reservoir was the only oxygenated reservoir to experience increased epilimnetic sulfate concentrations at turnover following oxygenation, but this is likely a result of external loading, as its oxygenation system was ineffective in increasing hypolimnetic sulfate concentrations during the dry season. Because we have not observed benefits of the hypolimnetic oxygenation systems in improving water quality at turnover, we focused our interpretations on water quality changes observed during operation of the systems.

Solar Circulators The solar circulator installed at Site 1 of Almaden Lake failed to improve dissolved oxygen concentrations, and the lake's hypolimnion remained anoxic for much of the dry season. Despite this, methylmercury concentrations decreased and sulfate concentrations increased in the hypolimnion. The circulator was not as effective in these regards as the hypolimnetic oxygenation systems, and methylmercury concentrations in Almaden Lake commonly exceeded the TMDL of 1.5 ng/L. Though internal nutrient loading appeared to decrease, as evident by reductions in ammonia and total phosphorus in the hypolimnion, chlorophyll *a* concentrations increased following the installation of the circulator. This may be due to the corresponding increase in epilimnetic total phosphorus concentrations, perhaps a result of mechanical mobilization of profundal compounds into the photic zone. Though dissolved oxygen concentrations were unchanged by the circulators, it is plausible that redox conditions were sufficiently enhanced to prolong aerobic microbial respiration at the sediment-water interface. However, some of the observed changes in water quality may be a result of the circulator mechanically diluting compounds throughout the water column. This is supported by the observation of significantly decreased ORP in the epilimnion following the installation of the circulator, which may be caused by the mixing of reducing hypolimnetic waters into the photic zone.

8.4 Fish Tissue Results

This section describes trends in fish tissue mercury concentrations measured prior to and during the operation of the reservoir Hypolimnetic Oxygenation Systems. As described in Section 5.6:

Remediation Effectiveness Indicators (REIs) are samples designed to be sensitive measures of mercury exposure variability in space and time. In the Guadalupe River Watershed, based on recommendations from the Regional Board, we have chosen "age-1" largemouth bass ranging from 55 to 102 mm in length as the primary REIs [4]. Since largemouth bass spawn during the springtime, REI samples are collected during the summer sampling event to ensure adequate tissue mass for laboratory analysis. Largemouth bass within the REI size range collected during the springtime are likely to represent the previous year's cohort.

Target Fish (TL3A and TL3B) are defined as 50 to 350 mm trophic level 3 fish. These fish are collected to measure progress in attaining fish tissue objectives of 0.05 mg Hg/kg (wet weight) for 50-150 mm fish (TL3A), and 0.1 mg Hg/kg (wet weight) for 150-350 mm fish (TL3B). These targets are intended to be protective of piscivorous birds. Thus, trophic level 3 target fish are collected just before or during the avian breeding season (spring sampling event), and during the summer sampling event.

Adult largemouth bass (TL4) range from 102-350 mm. Though these fish do not serve as targets or REIs, abundant historical data exists. Future adult largemouth bass samples will be collected during the spring sampling event only, representing the cohort of REI fish measured during the previous summer. This data will serve to determine bioaccumulation rates that occur during the wet season, as well as to minimize extrapolation in length-standardization.

8.4.1 Trend Evaluation Method

Several extraneous variables confound interpretation of time-trends in fish tissue concentrations. The data indicated five variables that had a significant influence on mercury content. Time trends are interpreted using a multiple linear regression model to minimize the effects of covariance. Omitting extraneous terms that significantly affect fish tissue mercury concentration avoids introducing bias into the interpretation of mercury concentration trends over time. Accounting for confounding variables ensures that time trend interpretations are not the result of inconsistent sampling patterns. Models are reservoir-specific, and included four additional explanatory variables:

$$\begin{aligned}\text{Hg (mg/kg ww)}_t = \alpha &+ \beta_1 \text{Year}_t \\ &+ \beta_2 \text{Species} \\ &+ \beta_3 \text{Length (mm)}_t \\ &+ \beta_4 \text{Collection Season}_t + \epsilon\end{aligned}$$

This method of evaluation is consistent with other studies that have accounted for location, species, length, and collection season when inferring trends [44]. The adjusted R^2 value for the model including reservoir, year, species, length, and collection season was 0.72, meaning that these five variables accounted for 72% of the variation in fish tissue mercury concentration. Because trends were evaluated for each reservoir individually, we created separate models for each reservoir, removing the "reservoir" term.

Year Though fish are collected biannually, we are primarily interested in general trends in fish tissue mercury concentrations. To simplify data interpretation, trends are assessed on an annual scale. The effect size of the year variable is used for time trend evaluation. This variable is calculated as the slope of the year variable multiplied by plus or minus 1.96 standard errors (to yield a 95% confidence interval), multiplied by the number of years of treatment. This calculation gives the total range of mercury concentration change attributable to time, and therefore treatment.

Fish Species Biomagnification causes lower-trophic level fish like bluegill to contain significantly lower mercury concentrations than more predatory fish like largemouth bass ($p<0.001$). Adult crappie are trophically-similar to small to medium bass. Even within a trophic group, there may be significant differences between individual species. For this reason, fish species is controlled for in the model (Figure 42).

Fish Length Each fish species exhibits a significant relationship between fork length and mercury content. (Figure 43). Longer fish are older, and have experienced greater dietary exposure to methylmercury. Because methylmercury is retained in tissues, concentrations increase with fork length ($p<0.001$). It is unclear why bluegill collected in Calero Reservoir are an exception to this trend.

Collection Season Studies have documented seasonal variations in fish tissue mercury levels, with concentrations typically increasing during the wet season. Fish growth rate increases during the summer, enhancing somatic growth dilution. During the winter, fish catabolize their muscle tissue for energy, decreasing body mass and thus increasing mercury concentration [57]. Studies document a seasonal pulse in mercury concentrations in fish following destratification, when bioavailable mercury is introduced to the food web [71]. Finally, because most sunfish spawn in the spring months, young fish collected during the summer likely reflect a recently-hatched cohort, exacerbating the effects of age. Our data confirms these patterns, with significantly higher mercury concentrations measured during the wet season ($p<0.001$) (Figure 44).

Figure 42: Mercury Concentrations by Species (All Years)

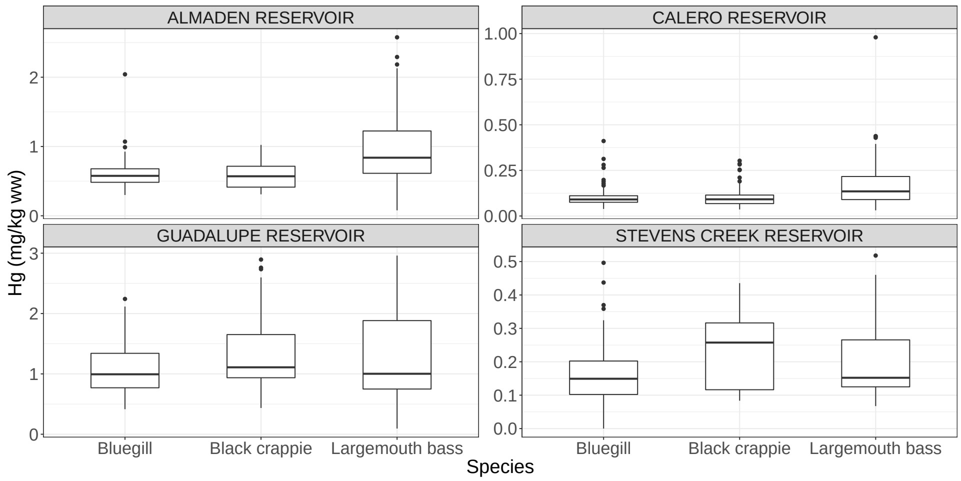
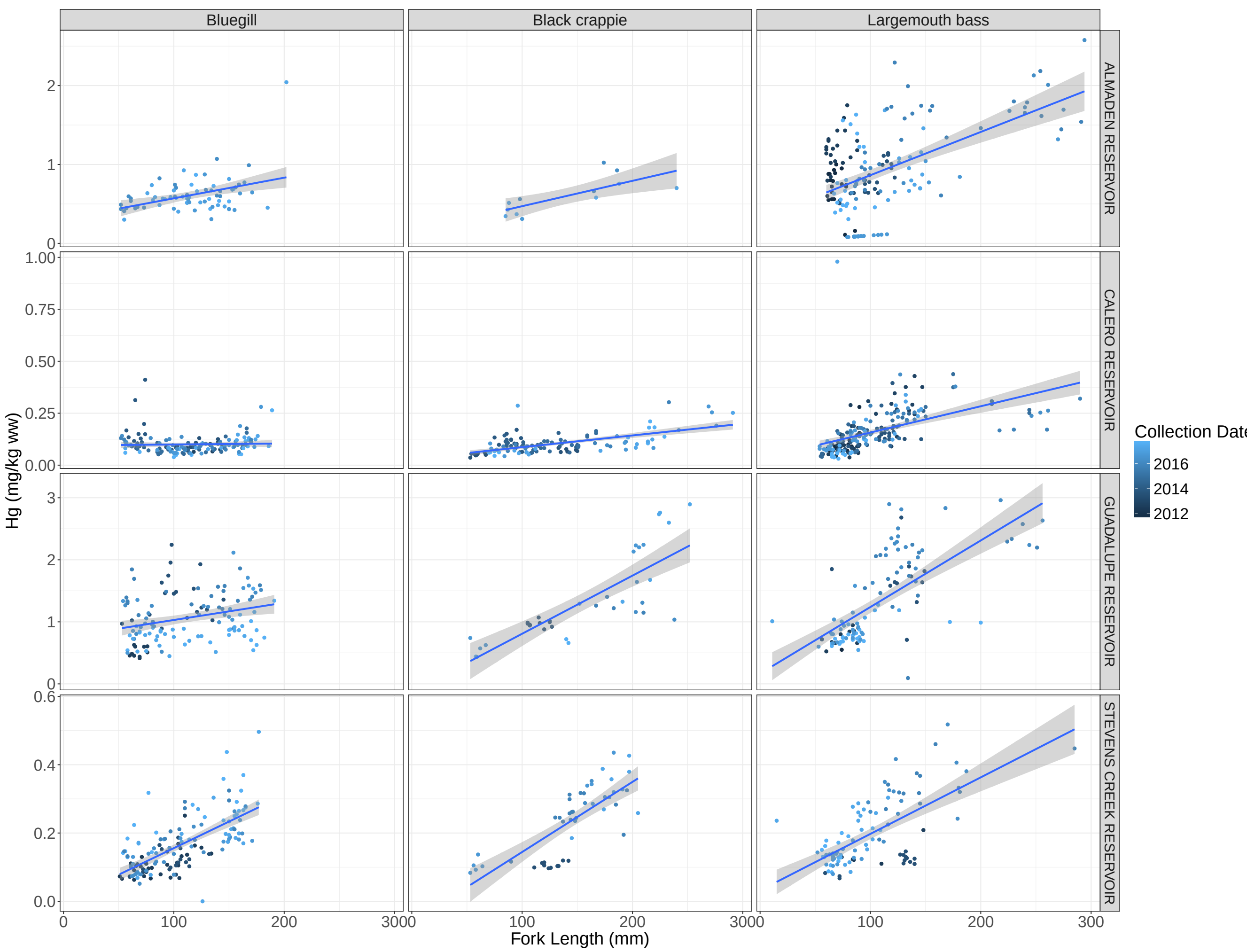


Figure 43: Mercury Concentrations by Fork Length



8.4.2 Results: Almaden Reservoir

Table 7 shows summary statistics for fish tissue mercury concentrations measured in Almaden Reservoir. Prior to the installation of the treatment systems, only largemouth bass were sampled. While eleven adult bass (TL4) were collected in 2013, most samples were REIs.

Figure 44: Mercury Concentrations by Season (All Fish Species)

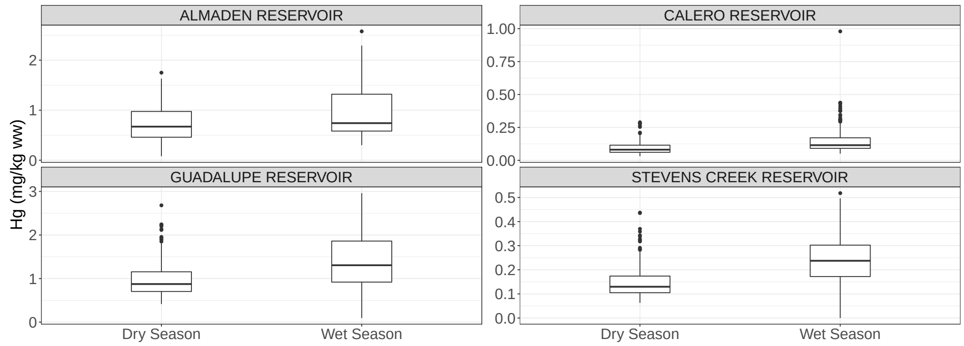
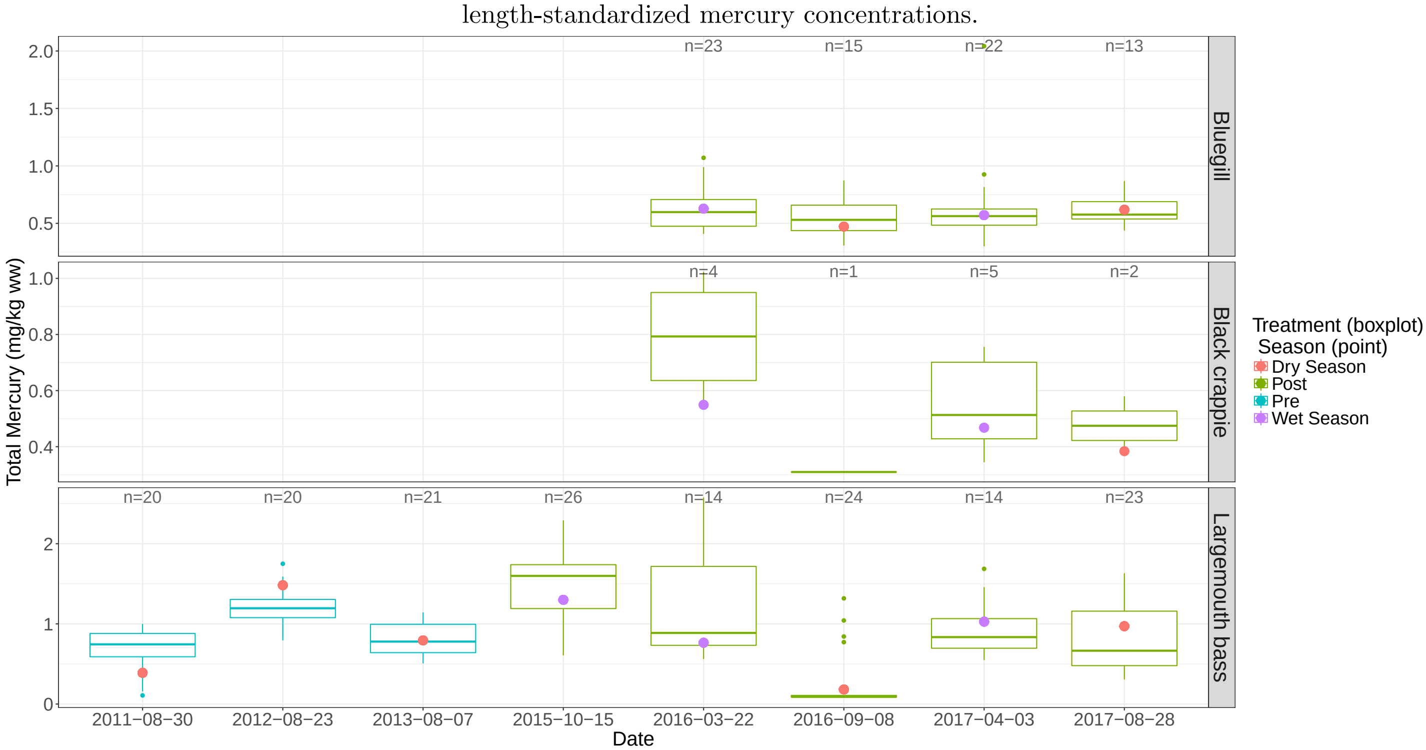


Table 7: Almaden Reservoir Fish Tissue Results

Year	Category	HOS	Count	Avg Hg (mg/kg)	SD (mg/kg)	CV	Avg Length (mm)	Length SD (mm)
2011	REI	Pre	20	0.7	0.24	0.34	69.9	7.82
2012	REI	Pre	20	1.21	0.23	0.19	69.8	9.23
2013	REI	Pre	10	0.68	0.12	0.17	89.2	10.71
2013	TL4	Pre	11	0.94	0.18	0.19	113.36	5.87
2015	REI	Post	5	0.91	0.18	0.2	94.2	1.3
2015	TL4	Post	21	1.61	0.36	0.23	179.48	59.44
2016	REI	Post	22	0.27	0.31	1.15	87.77	4.53
2016	TL3A	Post	32	0.56	0.16	0.29	101.53	32.25
2016	TL3B	Post	11	0.75	0.18	0.24	164.64	9.76
2016	TL4	Post	16	1.1	0.8	0.73	185.75	71.2
2017	REI	Post	21	0.83	0.43	0.52	79.67	8.41
2017	TL3A	Post	36	0.57	0.14	0.24	109.03	24.14
2017	TL3B	Post	6	0.87	0.58	0.67	189.33	29.98
2017	TL4	Post	16	0.91	0.33	0.36	132.56	13.43

Figure 45: Almaden Reservoir Fish Tissue Results

Boxplots reflect species-specific mercury concentrations from each sampling event. Points reflect 100 mm length-standardized mercury concentrations.



Pre and Post Treatment Figure 45 shows pre- and post-treatment total mercury concentrations of black crappie, bluegill, and largemouth bass. Please note that the largemouth bass facet displays both REI and adult fish.

Prior to treatment, REI fish in Almaden Reservoir had an average concentration of 0.90 mg/kg (SD=0.33, avg. length=73 mm). During treatment, REI fish contained significantly lower mercury concentrations ($p<0.001$), with an average concentration of 0.58 mg/kg (SD=0.46, avg. length=85 mm), though this is strongly influenced by anomalously low concentrations measured in 2016.

Adult largemouth bass had an average concentration of 0.94 mg/kg (SD=0.18, avg. length=113 mm) prior to treatment. In years when the oxygenation system was operated, we measured an average concentration of 1.27 mg/kg (SD=0.6, avg. length=169 mm), though these fish were significantly larger on average ($p<0.001$). Considering the effects of fork length and collection season on mercury concentration, adult largemouth bass still contained significantly higher mercury concentrations following oxygenation ($p<0.05$, ANOVA).

Time Trend Evaluation In Almaden Reservoir, the multiple regression model yielded no significant relationship between year and fish tissue mercury concentrations during oxygenation. The effect size of the collection year ranged from -0.31 to +0.19 mg/kg since the beginning of oxygenation (Figure 48).

8.4.3 Calero Reservoir

Table 8: Calero Reservoir Fish Tissue Results

Year	Category	HOS	Count	Avg Hg (mg/kg)	SD (mg/kg)	CV	Avg Length (mm)	Length SD (mm)
2011	REI	Pre	16	0.11	0.05	0.49	74.75	9.84
2012	REI	Pre	28	0.11	0.06	0.6	80.25	9.29
2012	TL3A	Pre	9	0.08	0.01	0.16	121.22	12.73
2012	TL4	Pre	12	0.28	0.1	0.35	128.75	11.93
2013	REI	Pre	8	0.11	0.04	0.37	77.38	9.66
2013	TL3A	Pre	49	0.11	0.07	0.62	94.31	31.87
2013	TL4	Pre	15	0.16	0.07	0.42	116.87	10.04
2014	REI	Post	19	0.11	0.05	0.46	79.16	14.64
2014	TL3A	Post	75	0.09	0.02	0.24	108.23	23.99
2014	TL3B	Post	10	0.13	0.04	0.27	161	6.07
2014	TL4	Post	20	0.24	0.08	0.33	151.9	43.47
2015	REI	Post	8	0.18	0.05	0.26	90.63	10.18
2015	TL3A	Post	16	0.1	0.02	0.19	106.88	28.9
2015	TL3B	Post	7	0.13	0.08	0.58	177	25.79
2015	TL4	Post	11	0.22	0.05	0.22	172.36	64.03
2016	REI	Post	21	0.12	0.04	0.31	77.67	12.78
2016	TL3A	Post	30	0.09	0.03	0.28	99.2	28.99
2016	TL3B	Post	18	0.14	0.07	0.5	195.89	41.92
2016	TL4	Post	13	0.24	0.09	0.39	156.23	58.24
2017	REI	Post	22	0.13	0.2	1.56	75.23	11.38
2017	TL3A	Post	28	0.08	0.04	0.58	96.54	21.46
2017	TL3B	Post	26	0.13	0.05	0.41	190.23	31.32
2017	TL4	Post	8	0.25	0.07	0.3	132.75	10.57

Table 8 shows summary statistics for fish tissue mercury concentrations measured in Calero Reservoir. Prior to the installation of the treatment systems, TL3A, REI, and TL4 fish were collected.

Pre and Post Treatment Figure 46 shows pre- and post-treatment total mercury concentrations of black crappie, bluegill, and largemouth bass.

Figure 46: Calero Reservoir Fish Tissue Results

Boxplots reflect species-specific mercury concentrations from each sampling event. Points reflect 100 mm length-standardized mercury concentrations.

Prior to treatment, REI fish in Calero Reservoir had an average concentration of 0.1 mg/kg (SD=0.06, avg. length=78 mm). During treatment, REI fish contained an average concentration of 0.14 mg/kg (SD=0.13, avg. length=80 mm). The difference between pre-and post-treatment mercury concentrations was not statistically significant.

Adult largemouth bass had an average concentration of 0.21 mg/kg (SD=0.1, avg. length=122 mm) prior to treatment. In years when the oxygenation system was operated, adult largemouth bass contained an average concentration of 0.24 mg/kg (SD=0.7, avg. length=154 mm). Considering the effects of fork length and collection season on mercury concentration, there was no significant difference between post- and pre-treatment concentrations.

Prior to treatment, TL3A fish had an average concentration of 0.1 mg/kg (SD=0.06, avg. length=98 mm). During treatment, REI fish contained an average concentration of 0.09 mg/kg (SD=0.03, avg. length=106 mm). The difference between pre-and post-treatment mercury concentrations was not statistically significant.

Time Trend Evaluation During the period of oxygenation, the effect size of the collection year ranged from -0.02 to +0.04 mg/kg in Calero Reservoir (Figure 48). The multiple regression model yielded no significant relationship between year and fish tissue mercury concentrations during system operation.

8.4.4 Guadalupe Reservoir

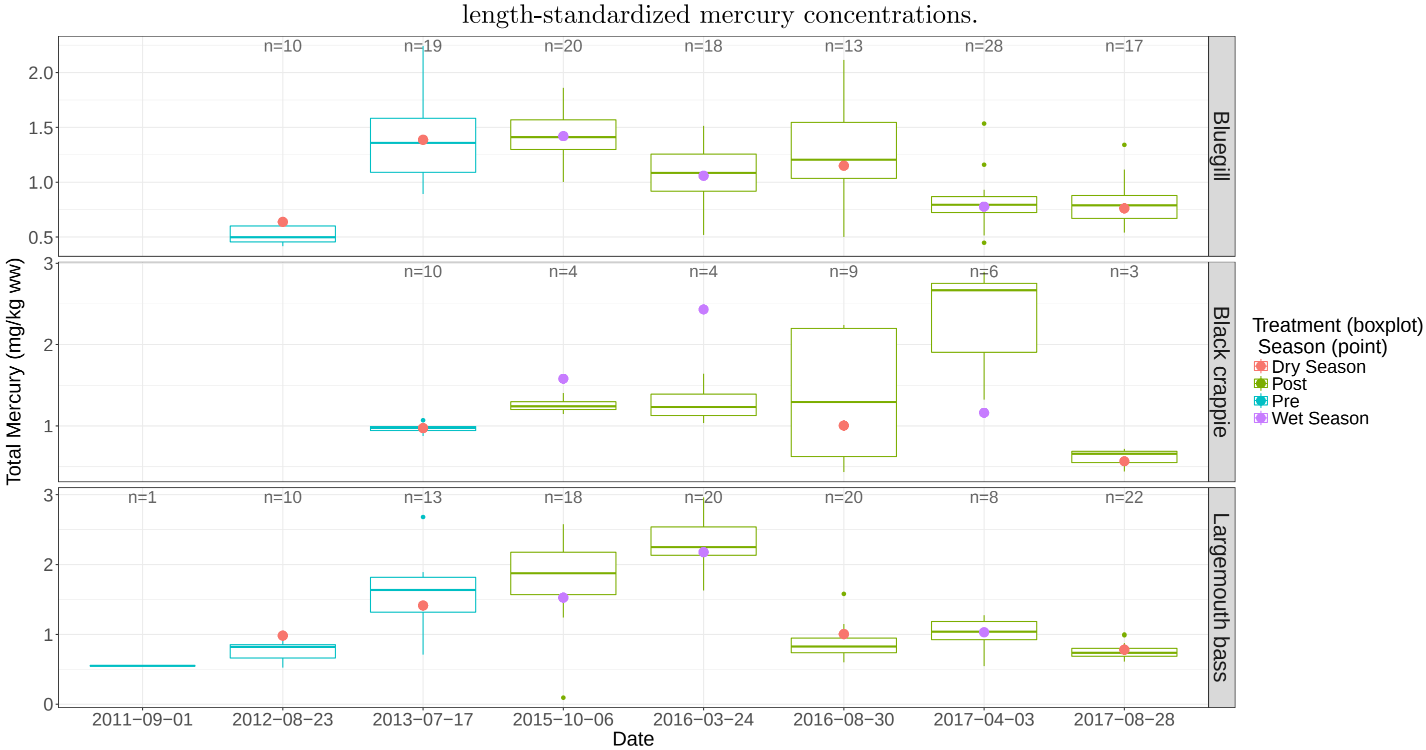
Table 9 shows summary statistics for fish tissue mercury concentrations measured in Guadalupe Reservoir. Prior to the installation of the treatment systems, TL3A, REI, and TL4 fish were collected.

Table 9: Guadalupe Reservoir Fish Tissue Results

Year	Category	HOS	Count	Avg Hg (mg/kg)	SD (mg/kg)	CV	Avg Length (mm)	Length SD (mm)
2011	REI	Pre	1	0.55	NA	NA	74	NA
2011	TL3A	Pre	8	0.81	0.2	0.25	73.5	12.06
2012	REI	Pre	10	0.77	0.13	0.17	77.8	10.44
2012	TL3A	Pre	10	0.52	0.08	0.16	67.8	5.73
2013	REI	Pre	3	1.1	0.65	0.6	59	5.2
2013	TL3A	Pre	29	1.25	0.37	0.29	107.48	20.81
2013	TL4	Pre	10	1.66	0.49	0.29	133.5	10.55
2015	REI	Post	1	1.54	NA	NA	95	NA
2015	TL3A	Post	16	1.4	0.22	0.16	101.81	37.2
2015	TL3B	Post	8	1.4	0.23	0.16	175.5	16.45
2015	TL4	Post	17	1.82	0.57	0.32	152.59	49.07
2016	REI	Post	20	0.93	0.26	0.28	80.6	10.73
2016	TL3A	Post	25	0.95	0.28	0.29	84.56	31.41
2016	TL3B	Post	19	1.56	0.44	0.28	182	26.08
2016	TL4	Post	19	2.35	0.33	0.14	146.84	43.61
2017	REI	Post	24	0.75	0.11	0.15	81	9.51
2017	TL3A	Post	32	0.73	0.14	0.19	95.94	28.78
2017	TL3B	Post	22	1.31	0.75	0.57	183.05	28.64
2017	TL4	Post	5	1.13	0.13	0.11	141.8	42.39

Figure 47: Guadalupe Reservoir Fish Tissue Results

Boxplots reflect species-specific mercury concentrations from each sampling event. Points reflect 100 mm length-standardized mercury concentrations.



Pre and Post Treatment Figure 47 shows pre- and post-treatment total mercury concentrations of black crappie, bluegill, and largemouth bass.

Prior to treatment, REI fish in Guadalupe Reservoir had an average concentration of 0.82 mg/kg (SD=0.32, avg. length=74 mm). During treatment, REI fish contained an average concentration of 0.94 mg/kg (SD=0.28, avg. length=82 mm). Considering the effects of fork length and collection season on mercury concentration, there was no significant difference between post- and pre-treatment concentrations.

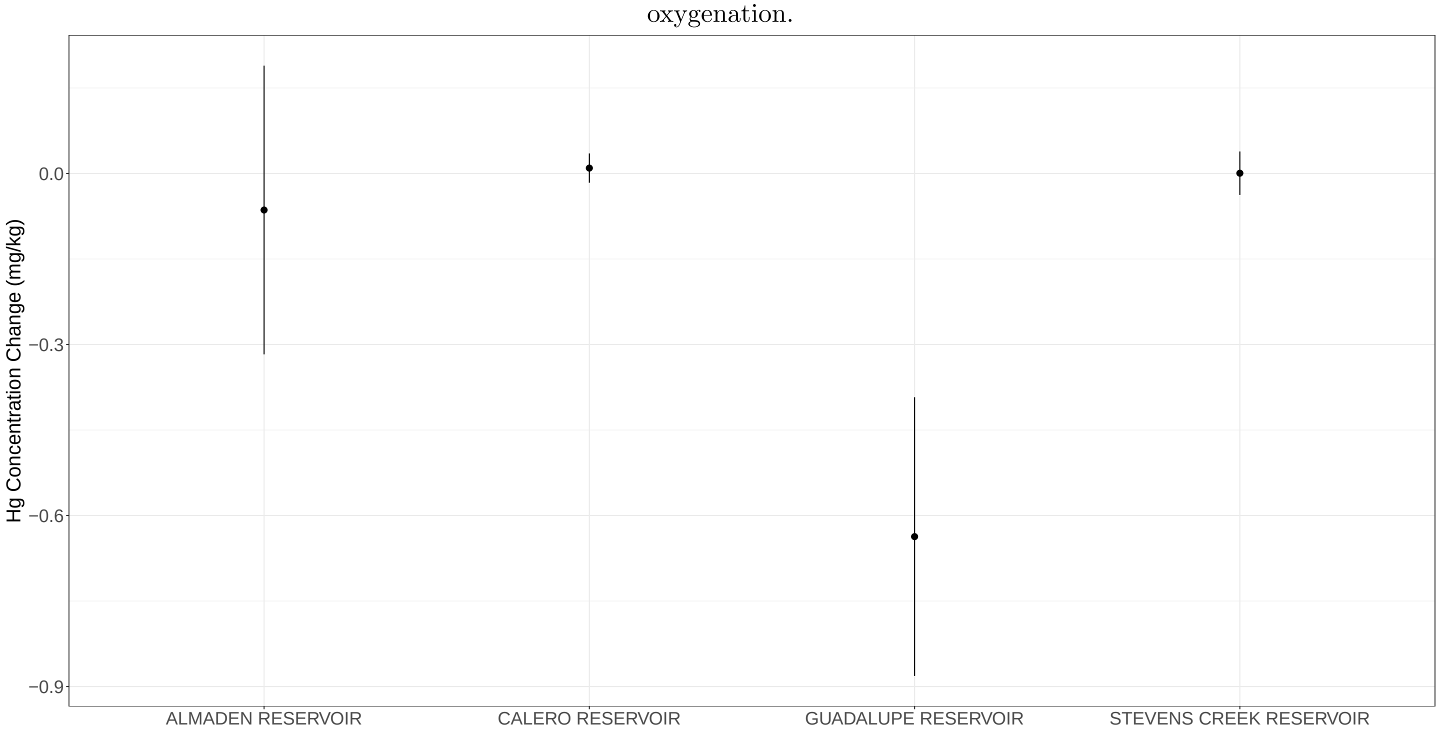
Adult largemouth bass had an average concentration of 1.70 mg/kg (SD=0.49, avg. length=134 mm) prior to treatment. In years when the oxygenation system was operated, we measured an average concentration of 2.03 mg/kg (SD=0.56, avg. length=147 mm). Considering the effects of fork length and collection season on mercury concentration, there was no significant difference between post- and pre-treatment concentrations.

Prior to treatment, TL3A fish had an average concentration of 1.06 mg/kg (SD=0.45, avg. length=97 mm). During treatment, TL3A fish contained an average concentration of 1.00 mg/kg (SD=0.34, avg. length=91 mm). Considering the effects of fork length, species, and collection season on mercury concentration, there was no significant difference between post- and pre-treatment concentrations.

Time Trend Evaluation Though fish tissue mercury concentrations have not improved relative to concentrations measured prior to the operation of the oxygenation system in Guadalupe Reservoir, the multiple regression model yielded a significant declining trend occurring during system operation ($p<0.001$). The effect size of the collection year ranged from -0.4 to -0.8 mg/kg in Guadalupe Reservoir (Figure 48). This is calculated as slope estimate for the multiple regression model's year variable (-0.2 mg/kg/yr following the initiation of treatment) plus or minus 1.96 standard errors (to yield a 95% confidence interval), multiplied by the number of years of system operation (3). If the current trend in mercury reduction of 0.2 mg/kg/year continues, TL3A and TL3B fish (on average) could reach their TMDL targets on the order of a half-decade, but this is difficult to predict due to high inter- and intra-annual variation.

Figure 48: Effect Size Ranges of Year on Fish Tissue Mercury Concentration

This plot shows the effect ranges of the year variable on mercury concentrations of reservoir fish, calculated using the multiple regression mofdel described in section, beginning at the initiation of oxygenation 8.4.1. Effect size ranges are calculated as the slope estimate for the year term plus or minus 1.96 standard errors (95% CI), multiplied by the number of years of oxygenation. This represents the total effect that time has had on mercury concentration since beginning oxygenation.



8.4.5 Almaden Lake

The Coordinated Monitoring Program collected fish tissue samples from Almaden Lake in 2012, 2013, and 2016. Please see AECOM's *Final Guadalupe River Coordinated Monitoring Program 5-Year Report* for discussions of trends in fish tissue concentrations in Almaden Lake.

The report is available on the Regional Board's website [3].

8.4.6 Reference Site: Stevens Creek Reservoir

Table 10: Stevens Creek Reservoir Fish Tissue Results

Year	Category	HOS	Count	Avg Hg (mg/kg)	SD (mg/kg)	CV	Avg Length (mm)	Length SD (mm)
2012	REI	Pre	4	0.13	0.01	0.05	79	6.38
2012	TL3A	Pre	29	0.11	0.04	0.38	86.07	22.56
2012	TL4	Pre	2	0.16	0.07	0.44	129	26.87
2013	REI	Pre	3	0.07	0.01	0.1	67.67	7.51
2013	TL3A	Pre	31	0.11	0.03	0.23	104.23	22.61
2013	TL4	Pre	10	0.13	0.01	0.09	133.1	4.31
2015	TL3A	Post	22	0.23	0.06	0.28	115.91	31.39
2015	TL3B	Post	6	0.27	0.06	0.21	172.67	20.01
2015	TL4	Post	14	0.33	0.08	0.23	149.36	45.28
2016	REI	Post	20	0.14	0.05	0.34	73.75	12.45
2016	TL3A	Post	33	0.13	0.05	0.38	91.3	31.4
2016	TL3B	Post	18	0.28	0.07	0.25	166.33	11.43
2016	TL4	Post	11	0.34	0.09	0.28	141.18	30.2
2017	REI	Post	30	0.17	0.06	0.36	77.5	14.22
2017	TL3A	Post	30	0.2	0.09	0.45	103.6	35.31
2017	TL3B	Post	20	0.3	0.08	0.29	169.5	15.37
2017	TL4	Post	3	0.26	0.05	0.18	110	5.57

Table 10 shows summary statistics for fish tissue mercury concentrations measured in Stevens Creek Reservoir. Prior to the installation of the treatment systems, TL3A, REI, and TL4 fish were collected.

Pre and Post Treatment Figure 49 shows pre- and post-treatment total mercury concentrations of black crappie, bluegill, and largemouth bass.

Prior to treatment, REI-sized fish in Stevens Creek Reservoir had an average concentration of 0.1 mg/kg (SD=0.02, avg. length=74 mm). During treatment, REI fish contained an average concentration of 0.17 mg/kg (SD=0.06, avg. length=80 mm). Considering the effects of fork length and collection season on mercury concentration, concentrations were significantly higher during operation of the oxygenation system ($p<0.001$, ANOVA).

Adult largemouth bass had an average concentration of 0.13 mg/kg (SD=0.03, avg. length=132 mm) prior to treatment. In years when the oxygenation system was operated, we measured an average concentration of 0.33 mg/kg (SD=0.08, avg. length=143 mm). Considering the effects of fork length and collection season on mercury concentration, concentrations were significantly higher during operation of the oxygenation system ($p<0.001$, ANOVA).

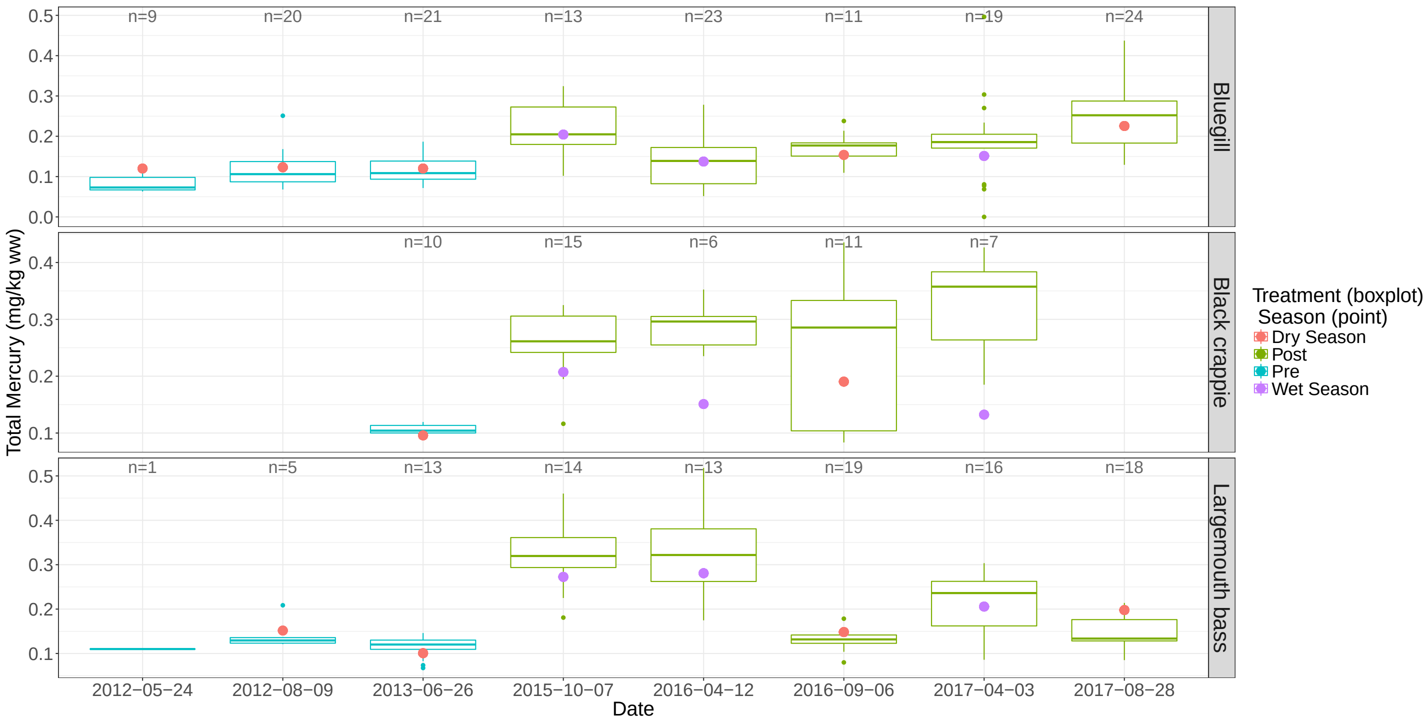
Prior to treatment, TL3A fish had an average concentration of 0.11 mg/kg (SD=0.03, avg. length=95 mm). During treatment, TL3A fish contained an average concentration of 0.17 mg/kg (SD=0.07, avg. length=103 mm). Considering the effects of fork length, species, and collection season on mercury concentration, concentrations were significantly higher during operation ($p<0.05$, ANOVA).

Time Trend Evaluation During years when the oxygenation system was operated, effect size of the collection year ranged from -0.04 to +0.04 mg/kg in Stevens Creek Reservoir (Figure 48). The multiple regression model

[3] https://www.waterboards.ca.gov/sanfranciscobay/water_issues/programs/TMDLs/guadaluperivermercurytmdl.shtml

Figure 49: Stevens Creek Reservoir Fish Tissue Results

Boxplots reflect species-specific mercury concentrations from each sampling event. Points reflect 100 mm length-standardized mercury concentrations.



yielded no significant relationship between year and fish tissue mercury concentrations during system operation.

8.4.7 Summary: Effectiveness in Improving Fish Tissue Mercury Concentrations

REI fish in Almaden Reservoir were the only collection category to improve significantly relative to pre-oxygenation conditions. However, this did not seem to translate throughout the food web, as data indicated a significant increase in mercury concentrations of adult largemouth bass and no significant declining trend occurring since the beginning of system operation. In Calero Reservoir, there was no significant difference in fish tissue mercury concentrations measured prior to and following the operation of the oxygenation system, and no significant trend in concentrations following installation. Though fish tissue mercury concentrations have not improved in Guadalupe Reservoir relative to pre-treatment levels, data indicates a significant declining trend occurring during operation. In Stevens Creek Reservoir, mercury concentrations of all fish categories increased during oxygenation. There was no significant trend in concentrations following the initial operation of the system. This may be due to significant increases in aqueous methylmercury concentrations measured in the photic zone during system operation, or due to unrelated changes in water chemistry or the food web occurring during system operation. The results of this study contrast with the Regional Board's prediction that "within months of deploying methylmercury production controls, mercury concentrations in age-1 fish will attain the TL3 wildlife target of 0.05 mg/kg" [4]. Though mercury concentrations in fish present many sources of variability, hypolimnetic oxygenation should be reconsidered as an efficient strategy for reducing fish tissue mercury concentrations. The District will continue to operate and evaluate treatment systems in hope that reductions are achieved over a longer time frame.

9 Mercury Loads from Points of Discharge

Section 9.4 of the Guadalupe River Watershed TMDL Staff Report requires the estimation of mercury loads at reservoir outlets:

> "*determine loads of mercury discharged annually to surface waters at the points of discharge.*"

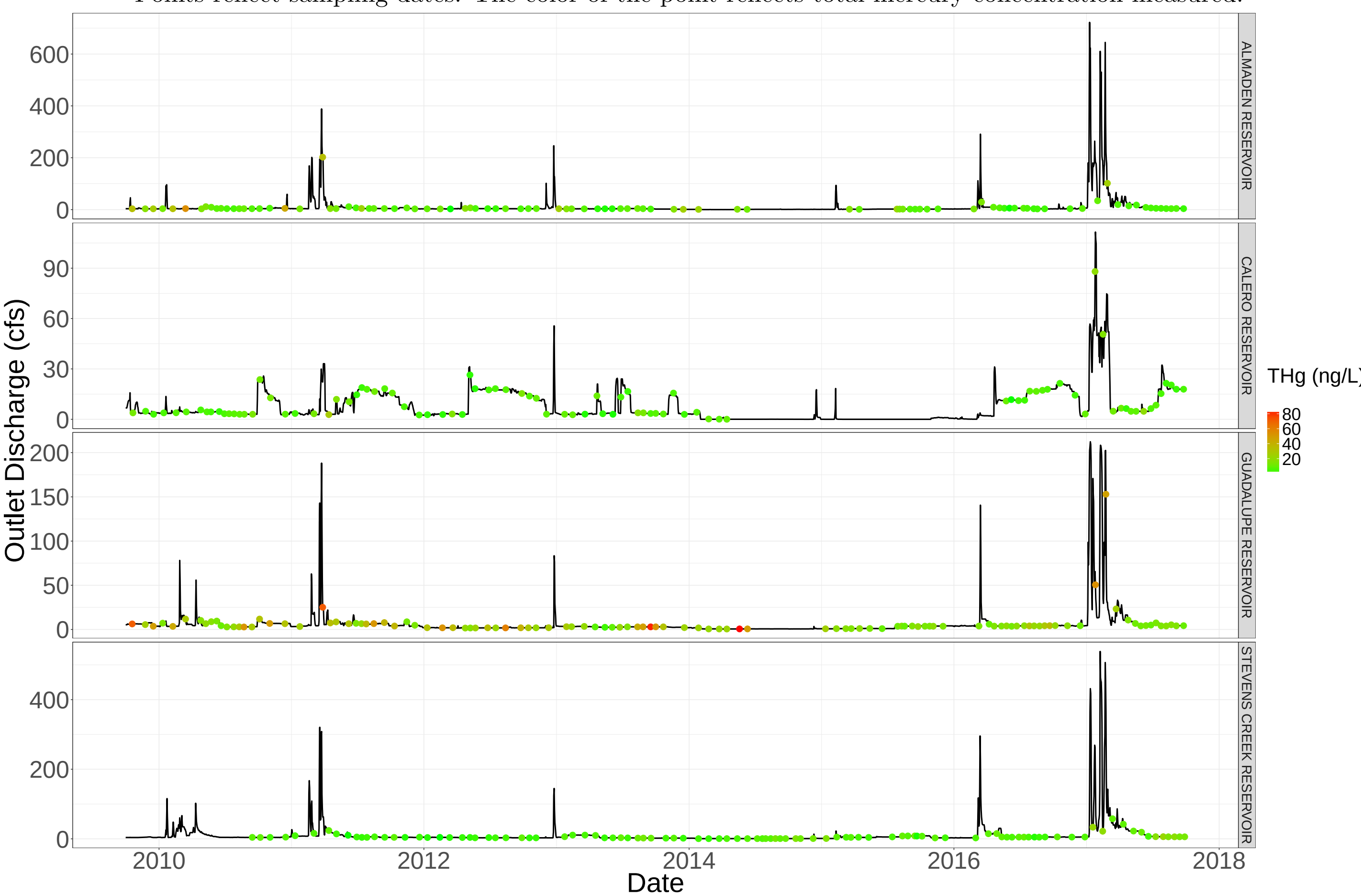
In this section, mercury and methylmercury loads are estimated by integrating reservoir outlet gauge data with outlet mercury and methylmercury concentrations measured bi-weekly to monthly. Loads are calculated as the total volume of water transferred between sampling events multiplied by mercury and methylmercury concentrations measured.

9.1 Results and Discussion

Tables 11 through 18 show annual total mercury and methylmercury loads calculated at reservoir outlets. Almaden and Stevens Creek Reservoirs spilled during the reporting period (Figure 2), so these estimates may slightly underrepresent true loading downstream. Samples were collected at regular intervals, so not all portions of the hydrograph were represented (Figure 50). Because mercury is thought to be transported primarily during high-intensity storm events, these load calculations likely underestimate actual loading.

Figure 50: Outlet Discharge, Sampling Dates, and Total Mercury Concentration

Points reflect sampling dates. The color of the point reflects total mercury concentration measured.



Figures 51 and 52 show the seasonal variations in annual loading from the reservoirs. Total mercury loading to downstream waters occurred primarily during the wet season, when concentrations were higher and more water was discharged from reservoir outlets. Drought conditions occurring from 2012 to 2016 dramatically reduced mercury loading from reservoirs. Surprisingly, Stevens Creek Reservoir accounted for more mercury loading downstream than Almaden or Calero reservoirs in Water Year 2017. While this was primarily a result of more water being discharged, it is notable that flow-weighted mean outlet concentrations were higher in Stevens Creek Reservoir. Stevens Creek Reservoir overlays Santa Clara Formation fluvial deposits, which are poorly indurated and highly erosive (Figure A.15). Though Stevens Creek Quarry has installed management structures to reduce fine-sediment input to the reservoir, outlet turbidity is significantly higher than the other reservoirs studied (Figure 53). Stevens Creek Reservoir's high outlet turbidity may enhance mercury loading, because mercury is commonly transported bound to sediments. High-intensity discharges from the upper Guadalupe River Watershed were poorly characterized, likely underrepresenting loading from Almaden and Guadalupe reservoirs. However, loading from reservoirs is governed more by reservoir operations (how much water is released) than by precipitation events. It is therefore feasible that much of the mercury transported into reservoirs during high-intensity storms is retained.

Flow-weighted mean concentrations are calculated as the total mercury load discharged per water year (in nanograms), divided by the total discharge (in liters). This reflects the average mercury concentration discharged downstream. Though flow-weighted mean total mercury concentrations have decreased in Guadalupe and Almaden reservoirs since 2010, high outlet discharge volumes have increased loading.

Figure 51: Dry and Wet Season Total Mercury Loads from Reservoir Outlets

During average flow years, the majority of methylmercury loading from reservoirs occurred during the dry season when production was highest. Guadalupe Reservoir discharges less methylmercury than expected due to its elevated outlet structure. In Almaden, Guadalupe, and Stevens Creek reservoirs, the hypolimnetic oxygenation systems significantly reduced outlet methylmercury concentrations, which reduced dry season loads. Methylmercury loading remains high from Calero Reservoir.

Figure 52: Dry and Wet Season Total Methylmercury Loads from Reservoir Outlets

Figure 53: Outlet Turbidity of Reservoirs and Lake

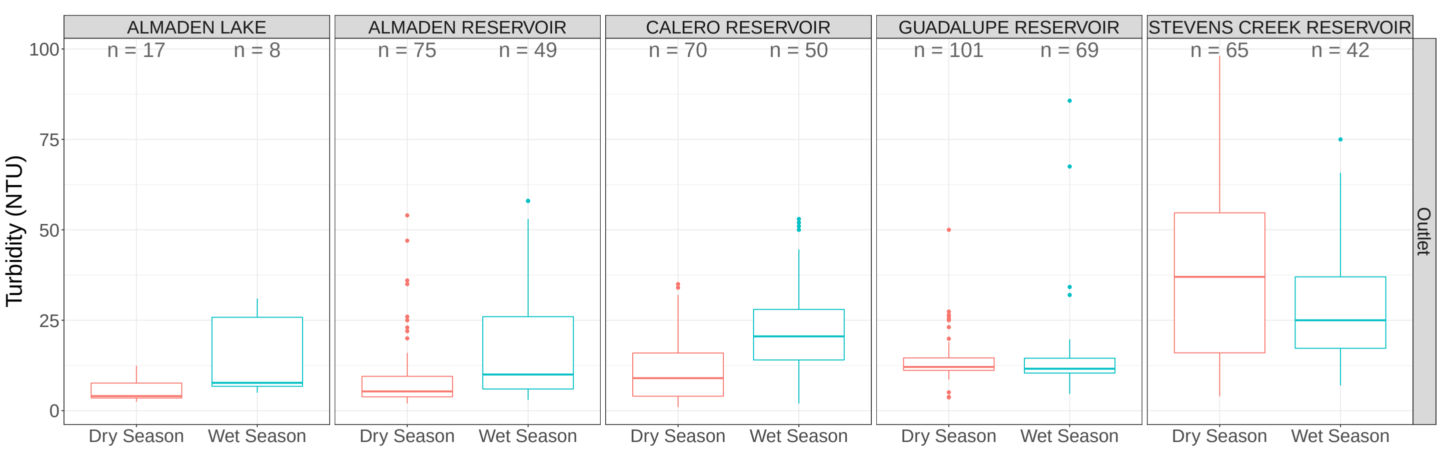


Table 11: Total Mercury Loads: Almaden Reservoir

Water Year	Hg Load (grams)	Million Gallons Discharged	Flow Weighted Mean Conc. (ng/L)
2010	87.47	1286.64	17.96
2011	254.95	4019.11	16.76
2012	25.69	941.90	7.21
2013	43.72	1415.96	8.16
2014	10.25	218.08	12.42
2015	22.19	546.60	10.72
2016	59.39	1609.63	9.75
2017	356.49	10330.66	9.12

Table 12: Total Methylmercury Loads: Almaden Reservoir

Water Year	MeHg Load (grams)	Million Gallons Discharged	Flow Weighted Mean Conc. (ng/L)
2010	5.89	1286.64	1.21
2011	5.52	4019.11	0.36
2012	3.08	941.90	0.86
2013	3.55	1415.96	0.66
2014	0.91	218.08	1.10
2015	3.50	546.60	1.69
2016	1.88	1609.63	0.31
2017	9.59	10330.66	0.25

Table 13: Total Mercury Loads: Calero Reservoir

Water Year	Hg Load (grams)	Million Gallons Discharged	Flow Weighted Mean Conc. (ng/L)
2010	31.06	1027.57	7.99
2011	97.77	2537.57	10.18
2012	50.12	2459.58	5.38
2013	45.67	1689.32	7.14
2014	10.67	466.00	6.05
2015	2.00	60.84	8.68
2016	41.32	1633.10	6.68
2017	166.41	4358.82	10.09

Table 14: Total Methylmercury Loads: Calero Reservoir

Water Year	MeHg Load (grams)	Million Gallons Discharged	Flow Weighted Mean Conc. (ng/L)
2010	8.79	1027.57	2.26
2011	26.22	2537.57	2.73
2012	9.93	2459.58	1.07
2013	8.02	1689.32	1.25
2014	0.16	466.00	0.09
2015	0.16	60.84	0.69
2016	8.34	1633.10	1.35
2017	9.13	4358.82	0.55

Table 15: Total Mercury Loads: Guadalupe Reservoir

Water Year	Hg Load (grams)	Million Gallons Discharged	Flow Weighted Mean Conc. (ng/L)
2010	184.06	1482.46	32.80
2011	289.73	2231.17	34.30
2012	72.12	611.11	31.18
2013	76.64	787.90	25.70
2014	40.18	294.17	36.08
2015	25.00	345.85	19.10
2016	77.16	1246.74	16.35
2017	551.32	4803.91	30.32

Table 16: Total Methylmercury Loads: Guadalupe Reservoir

Water Year	MeHg Load (grams)	Million Gallons Discharged	Flow Weighted Mean Conc. (ng/L)
2010	4.75	1482.46	0.85
2011	8.92	2231.17	1.06
2012	3.46	611.11	1.50
2013	5.05	787.90	1.69
2014	1.29	294.17	1.16
2015	3.95	345.85	3.02
2016	2.93	1246.74	0.62
2017	3.03	4803.91	0.17

Table 17: Total Mercury Loads: Stevens Creek Reservoir

Water Year	Hg Load (grams)	Million Gallons Discharged	Flow Weighted Mean Conc. (ng/L)
2010	91.52	2501.84	9.66
2011	74.22	3564.98	5.50
2012	11.31	887.47	3.37
2013	209.67	1418.75	39.04
2014	5.43	236.99	6.05
2015	24.32	962.00	6.68
2016	56.33	2424.99	6.14
2017	516.92	9027.85	15.13

Table 18: Total Methylmercury Loads: Stevens Creek Reservoir

Water Year	MeHg Load (grams)	Million Gallons Discharged	Flow Weighted Mean Conc. (ng/L)
2010	1.70	2501.84	0.18
2011	2.82	3564.98	0.21
2012	2.71	887.47	0.81
2013	1.95	1418.75	0.36
2014	0.31	236.99	0.35
2015	1.05	962.00	0.29
2016	1.29	2424.99	0.14
2017	4.05	9027.85	0.12

10 Recommendations

The District is committed to continuing its voluntary studies of methylmercury production and control measures, and will notify the Regional Board should any capital projects occur that may impede monitoring efforts. The District intends to continue operation of the methylmercury control systems, and does not plan to modify operational procedures during the next two-year reporting period.

The District provides the following recommendations for current and future studies of methylmercury control systems:

Current District Study

- The District requests the elimination of Sampling Sites 2-5 at Almaden Lake. Site one, containing the first solar circulator installed (included in this report), and the inlet and outlet sites are sufficient for assessing treatment system effectiveness.
- The District requests to discontinue manganese and iron sampling in reservoirs. No data was collected in Almaden Lake, Almaden Reservoir, Guadalupe Reservoir, and Stevens Creek Reservoir prior to the installation of the treatment systems, so there is no baseline to compare to. Additionally, this report demonstrates these analytes as poor predictors of redox potential.
- The District requests to discontinue outlet sampling at Almaden, Calero, and Stevens Creek reservoirs, because outlet samples were found to be statistically indistinguishable from samples collected in their hypolimnia.
- The District considers Special Study 1 to be complete. If the Regional Board is satisfied with the results of the comparative study presented in this document, which incorporate 10 years of monitoring data, the District requests to discontinue monitoring of the following analytes: Ammonia, Total Phosphorus, Nitrate, and Nitrite. The TMDL does not require monitoring of nutrient cycling, or effectiveness evaluation of treatment systems regarding internal nutrient loading.

Following approval from the Regional Board, the District will produce an updated plan describing future monitoring activities.

Statewide Mercury Control Program

- Consider dry-season methylmercury concentrations present in the photic zone when evaluating treatment system effectiveness. Fish foraging and metabolism decreases during the winter months, so dietary exposure to methylmercury is most significant during the dry season.
- Line diffuser systems may not be ideal systems for large or weakly-stratified reservoirs like Calero Reservoir. Oxygen retention appears to be more efficient in smaller reservoirs with high relative depths.
- Consider the effects of outlet structure on profundal compounds available to the photic zone during turnover. Different outlet structure elevations may impact compound movement during and after reservoir turnover. Additional studies with frequent monitoring during reservoir turnover may be needed to capture these effects. The District may conduct special studies related to the effects of variable outlet configurations in the future, as Calero Reservoir's outlet structure may be replaced or modified as part of the Calero Dam Seismic Retrofit Project.
- The variable foraging patterns of young largemouth bass lead to high variance in mercury concentrations, perhaps making them a poor short-term indicator of treatment system effectiveness. Less predatory fish should be used whenever possible in future studies mandated by the State Water Resources Control Board.

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A Maps

Figure A.1: Guadalupe River Watershed Location

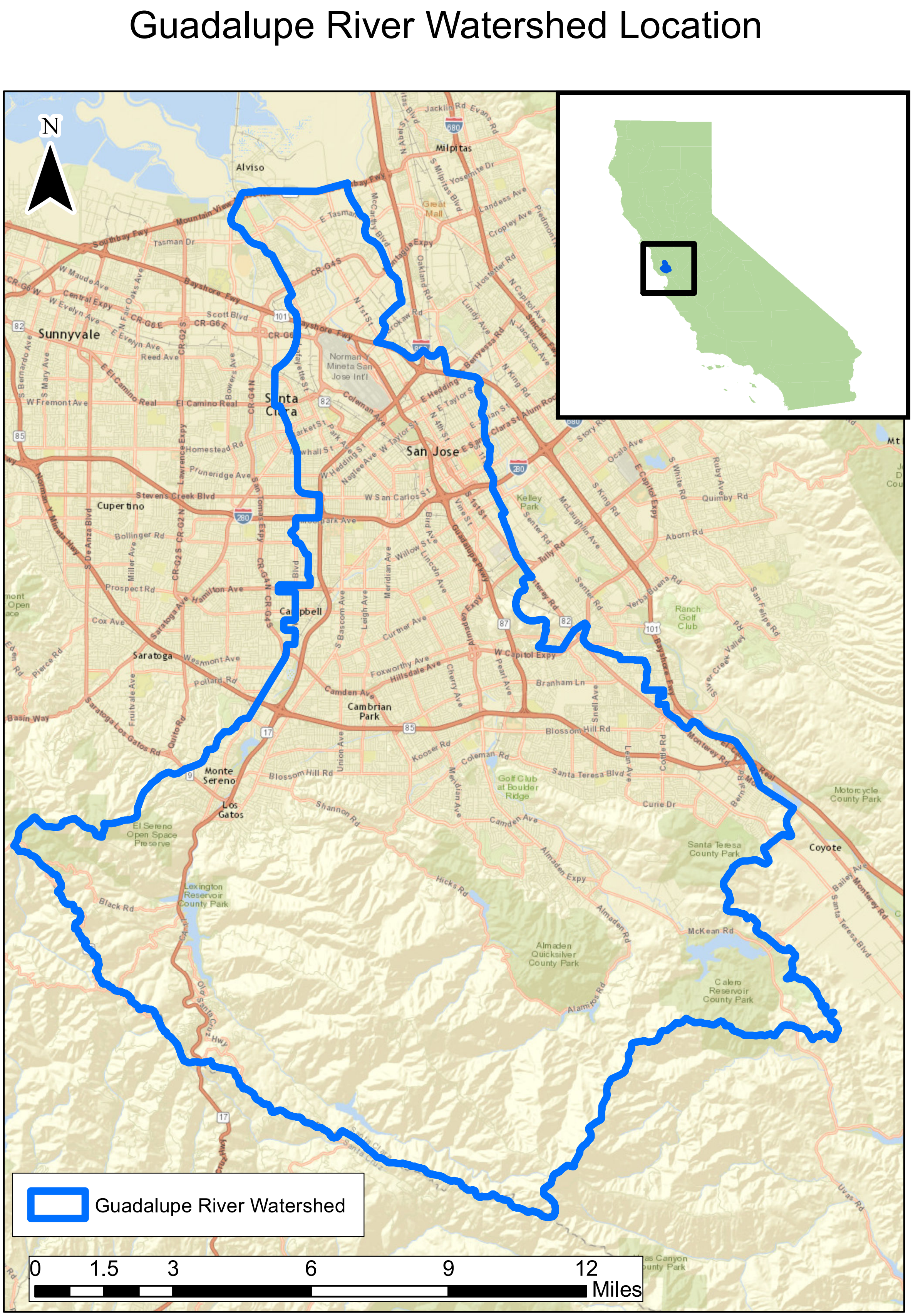


Figure A.2: Hydrologic Connectivity of Upper Guadalupe River Watershed

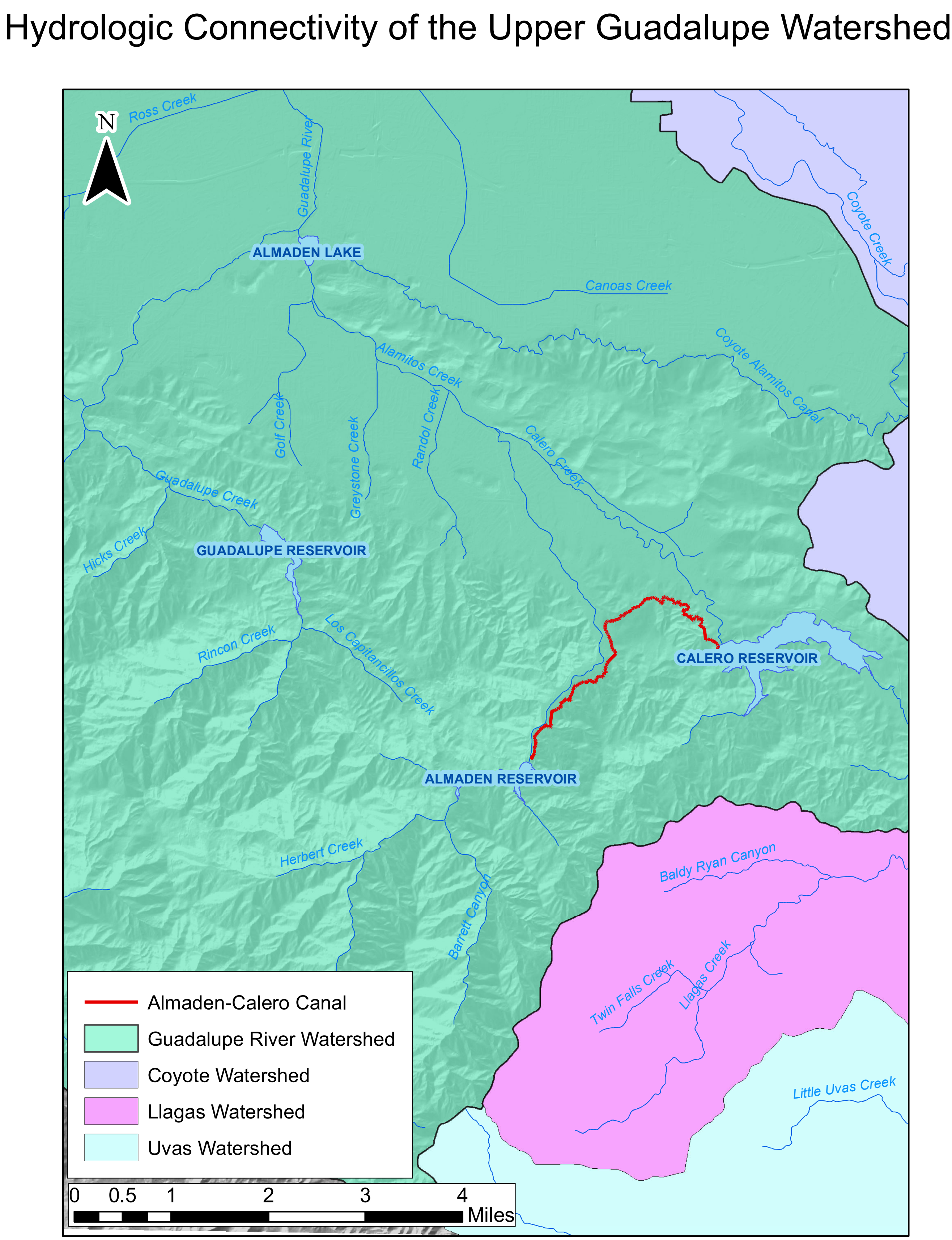
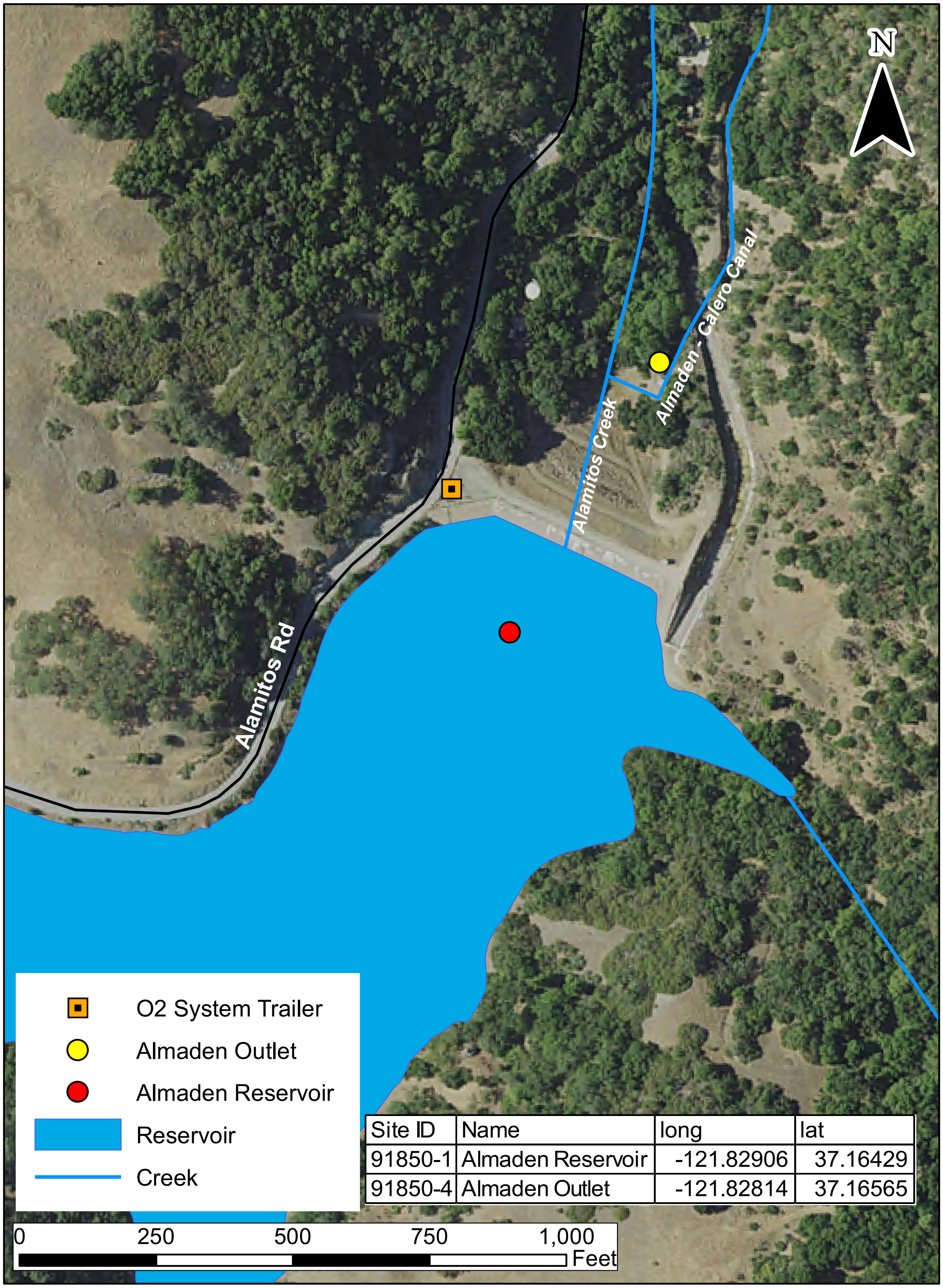


Figure A.3: Almaden Reservoir Sampling Sites

Almaden Reservoir Sampling Sites



Site ID	Name	long	lat
91850-1	Almaden Reservoir	-121.82906	37.16429
91850-4	Almaden Outlet	-121.82814	37.16565

Figure A.4: Calero Reservoir Sampling Sites

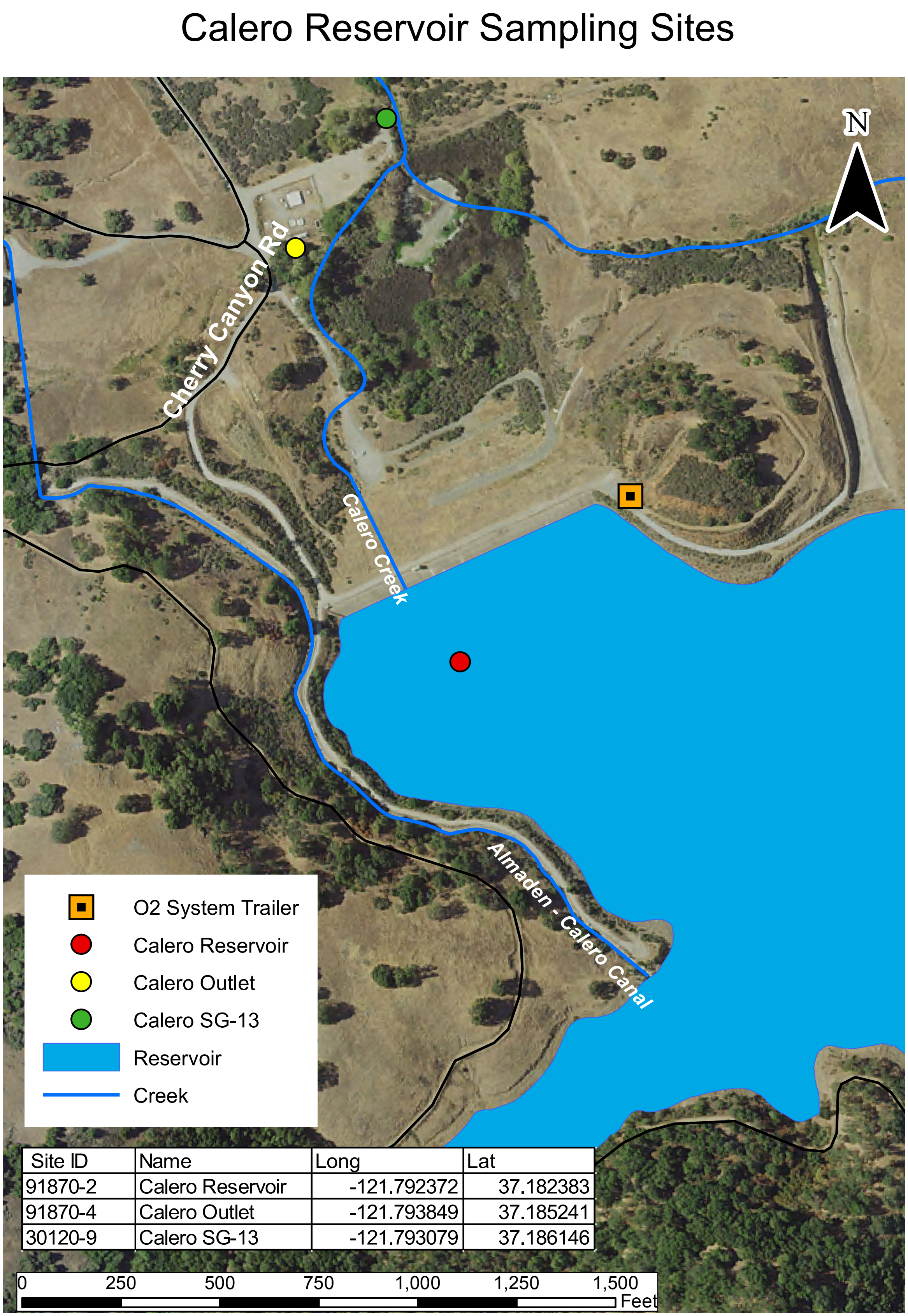
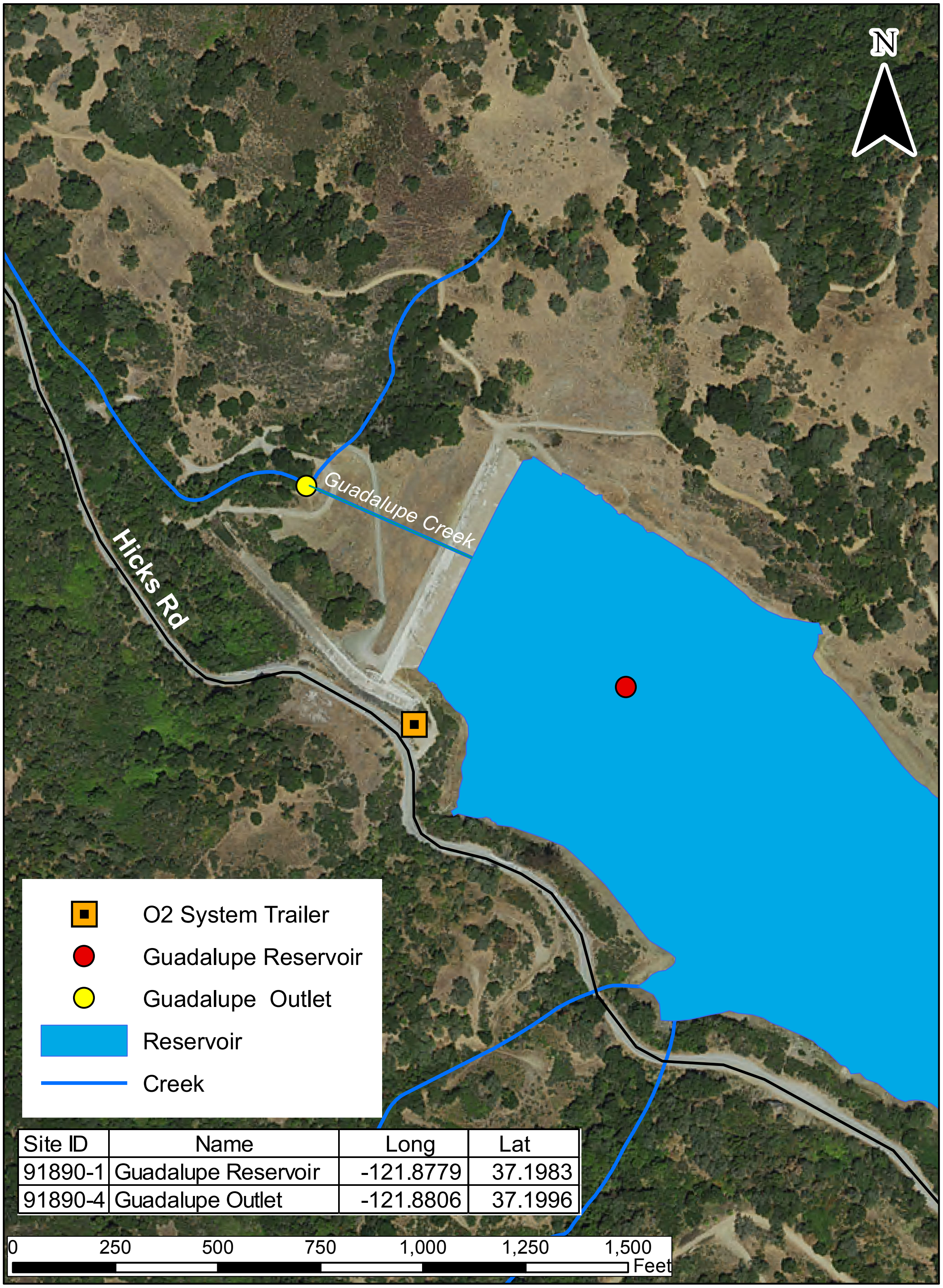


Figure A.5: Guadalupe Reservoir Sampling Sites

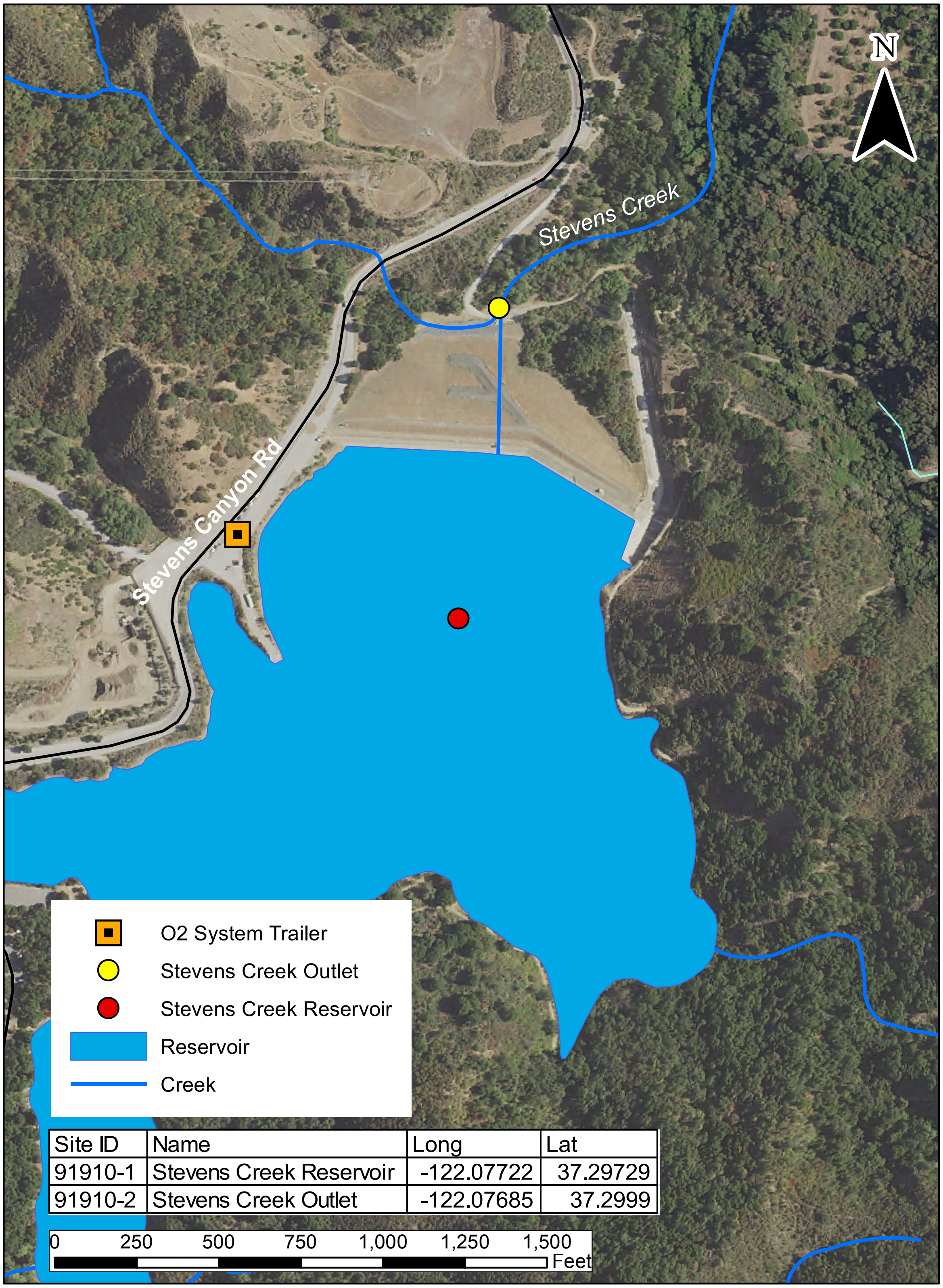
Guadalupe Reservoir Sampling Sites



Site ID	Name	Long	Lat
91890-1	Guadalupe Reservoir	-121.8779	37.1983
91890-4	Guadalupe Outlet	-121.8806	37.1996

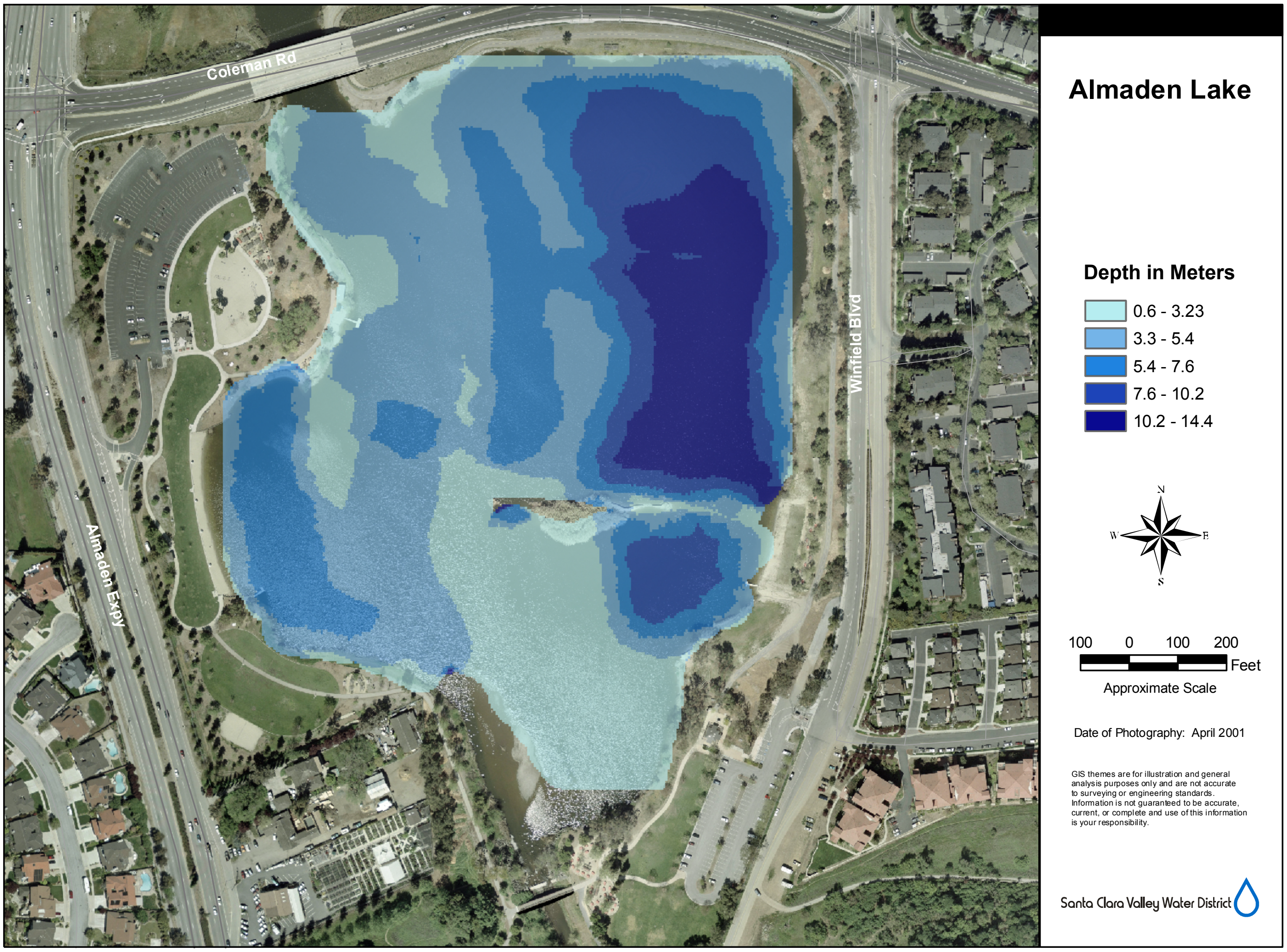
Figure A.6: Stevens Creek Reservoir Sampling Sites

Stevens Creek Reservoir Sampling Sites



Site ID	Name	Long	Lat
91910-1	Stevens Creek Reservoir	-122.07722	37.29729
91910-2	Stevens Creek Outlet	-122.07685	37.2999

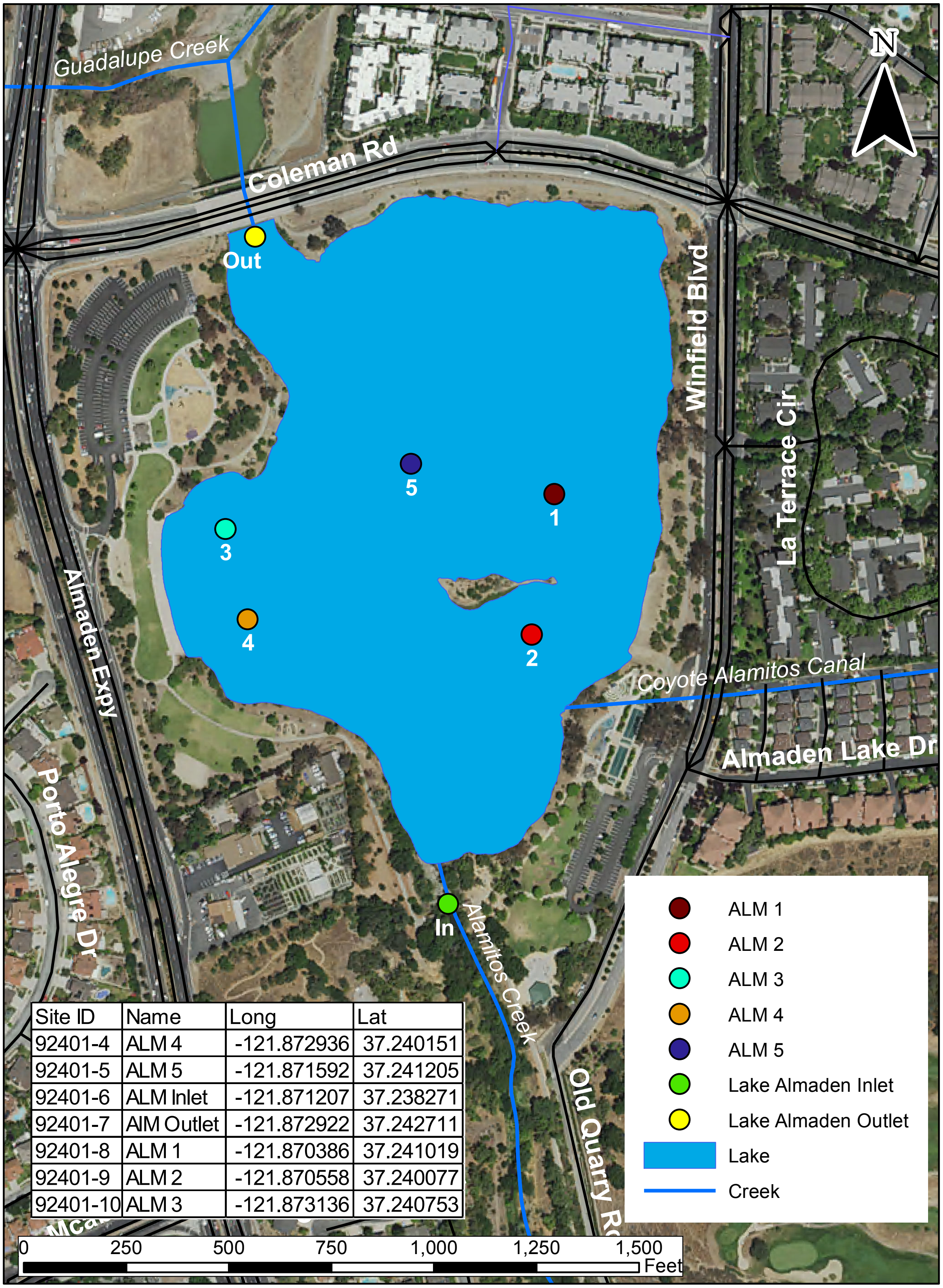
Figure A.7: Almaden Lake Bathymetry



26752043\2006_068\almaden.mxd 04/24/2006

Figure A.8: Almaden Lake Sampling Sites

Lake Almaden Sampling Sites



Site ID	Name	Long	Lat
92401-4	ALM 4	-121.872936	37.240151
92401-5	ALM 5	-121.871592	37.241205
92401-6	ALM Inlet	-121.871207	37.238271
92401-7	AlM Outlet	-121.872922	37.242711
92401-8	ALM 1	-121.870386	37.241019
92401-9	ALM 2	-121.870558	37.240077
92401-10	ALM 3	-121.873136	37.240753

Figure A.9: Almaden Lake and Alamitos Flashboard Dam

Figure A.10: Guadalupe Reservoir Slope Map

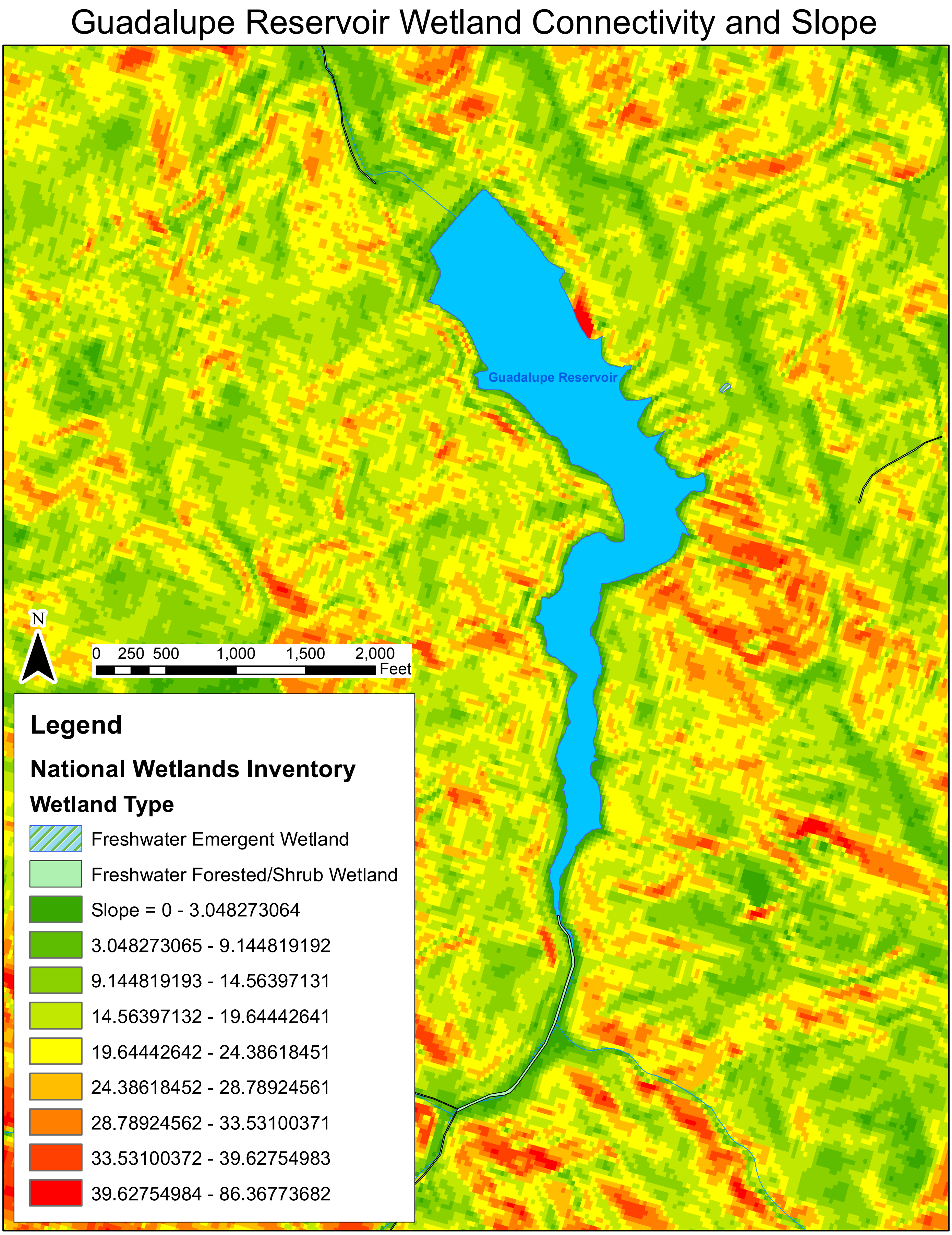


Figure A.11: Stevens Creek Reservoir Slope Map

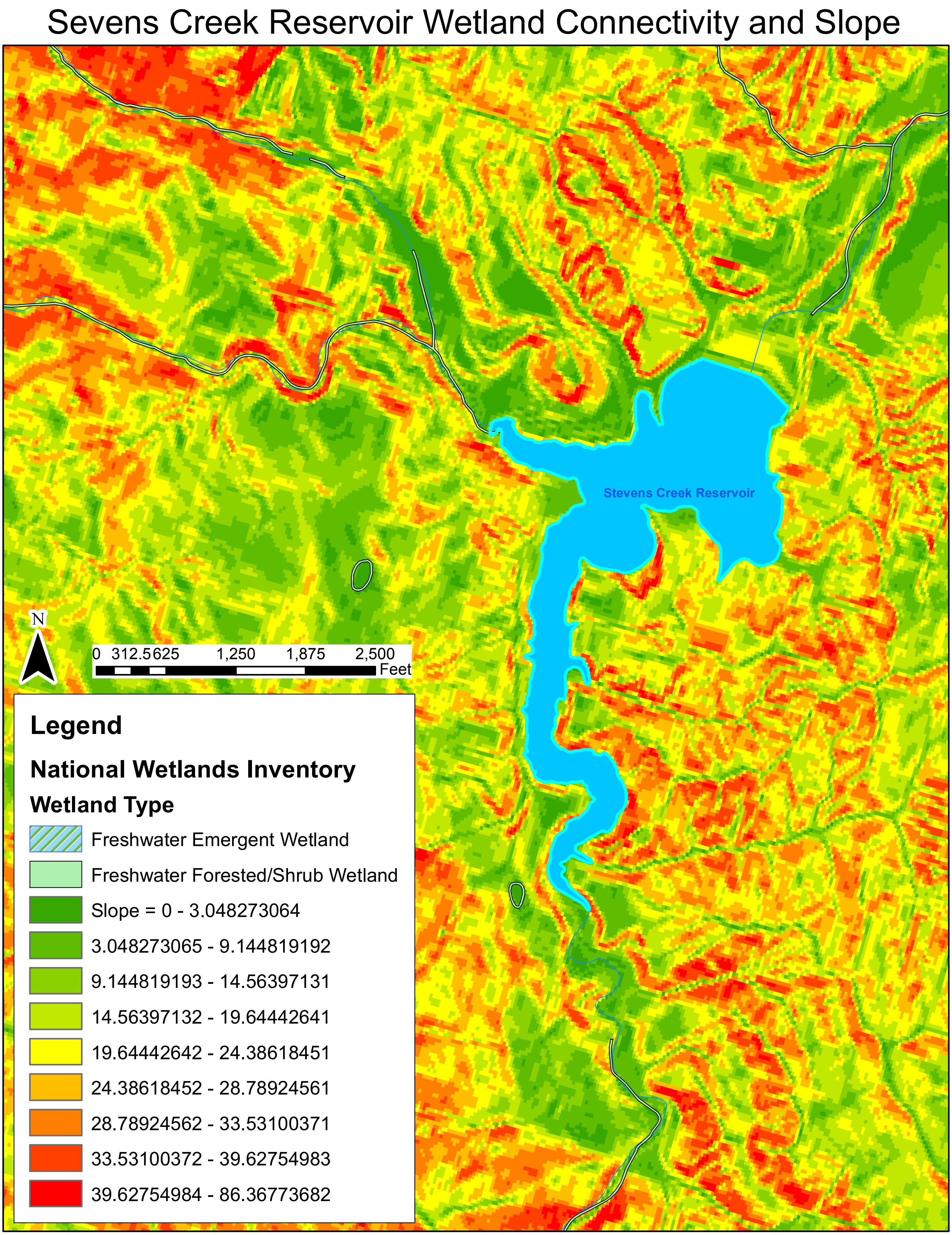


Figure A.12: Almaden Reservoir Wetland Connectivity

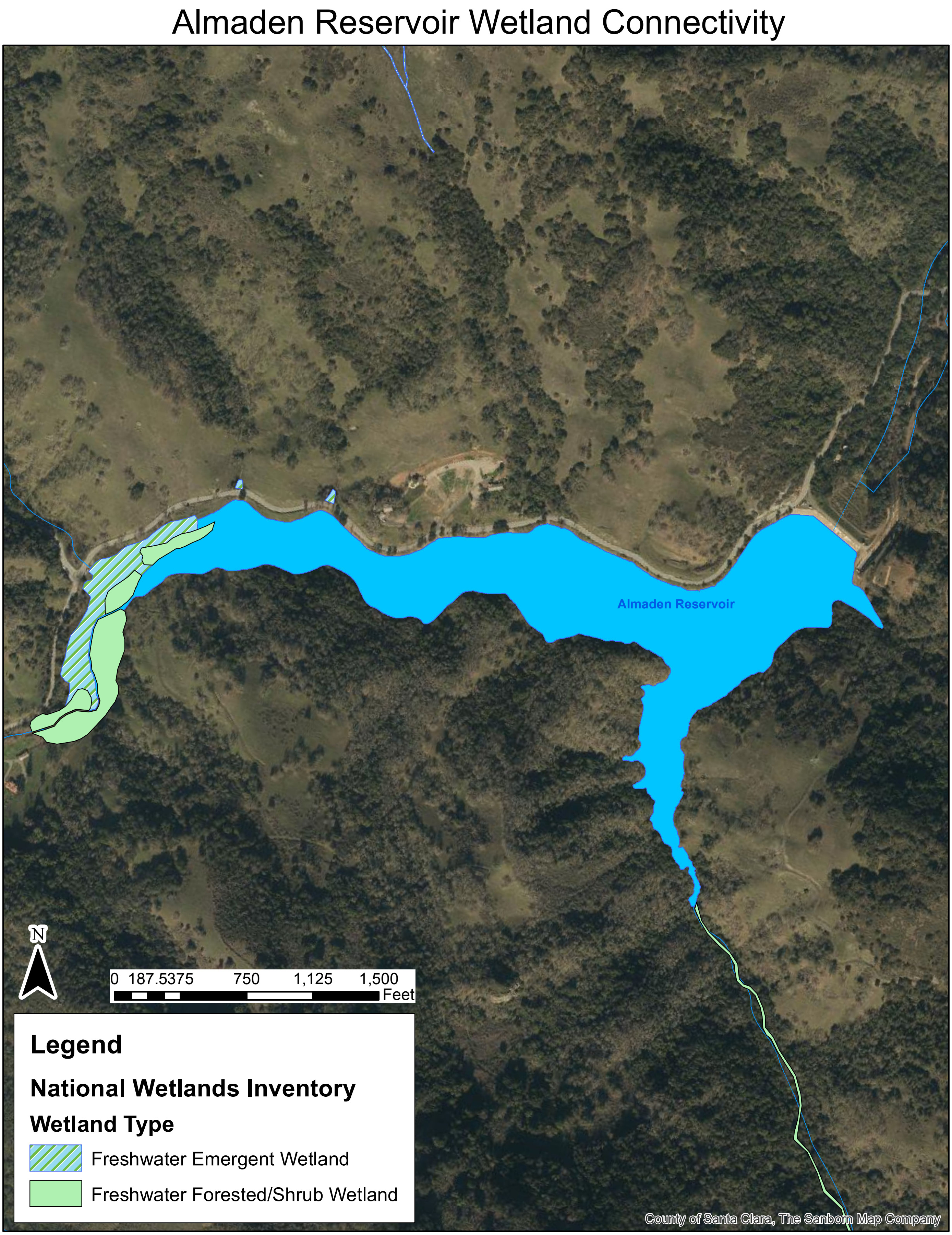


Figure A.13: Calero Reservoir Wetland Connectivity

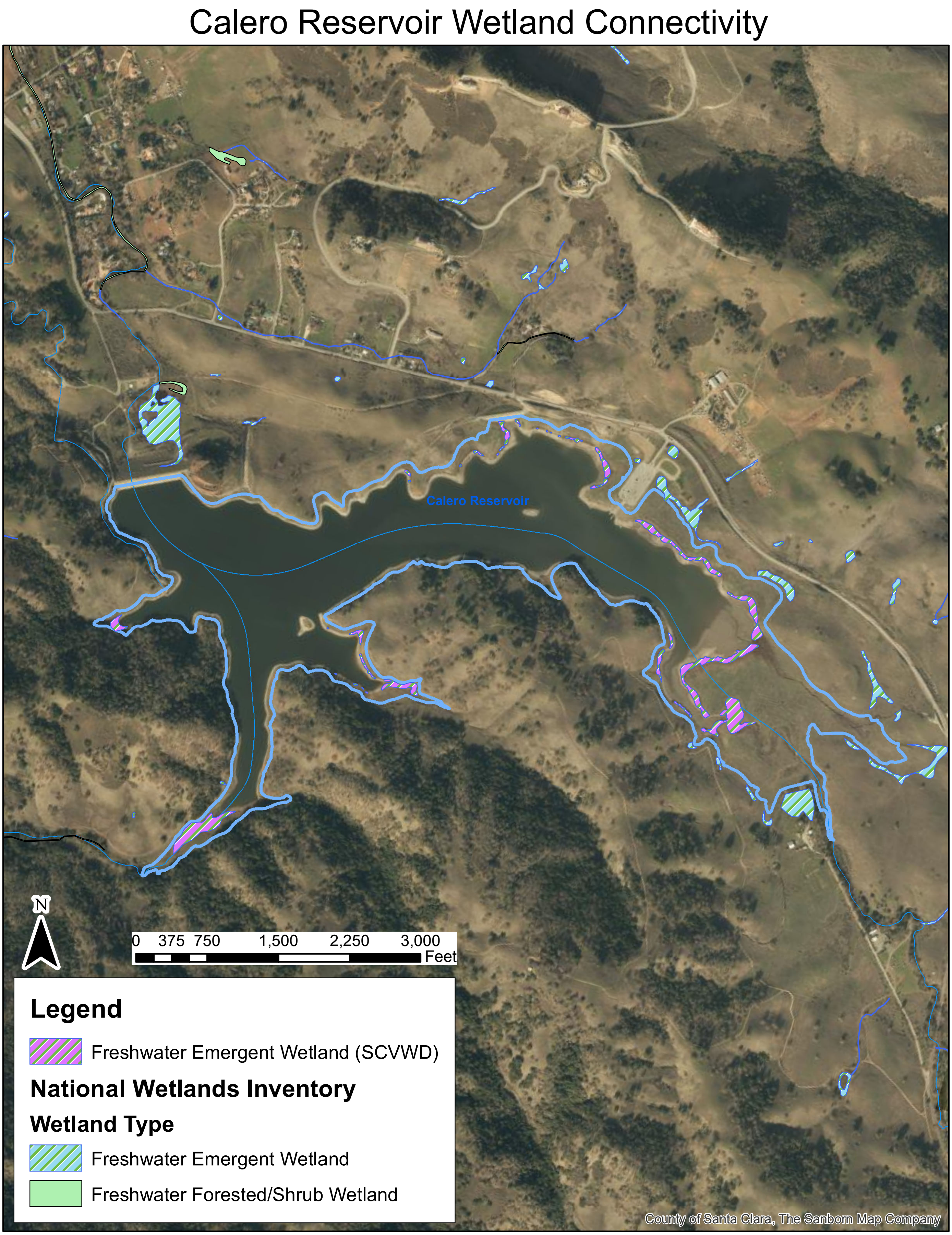


Figure A.14: Geologic Map of New Almaden Mining District

Geologic Map of New Almaden Mining District

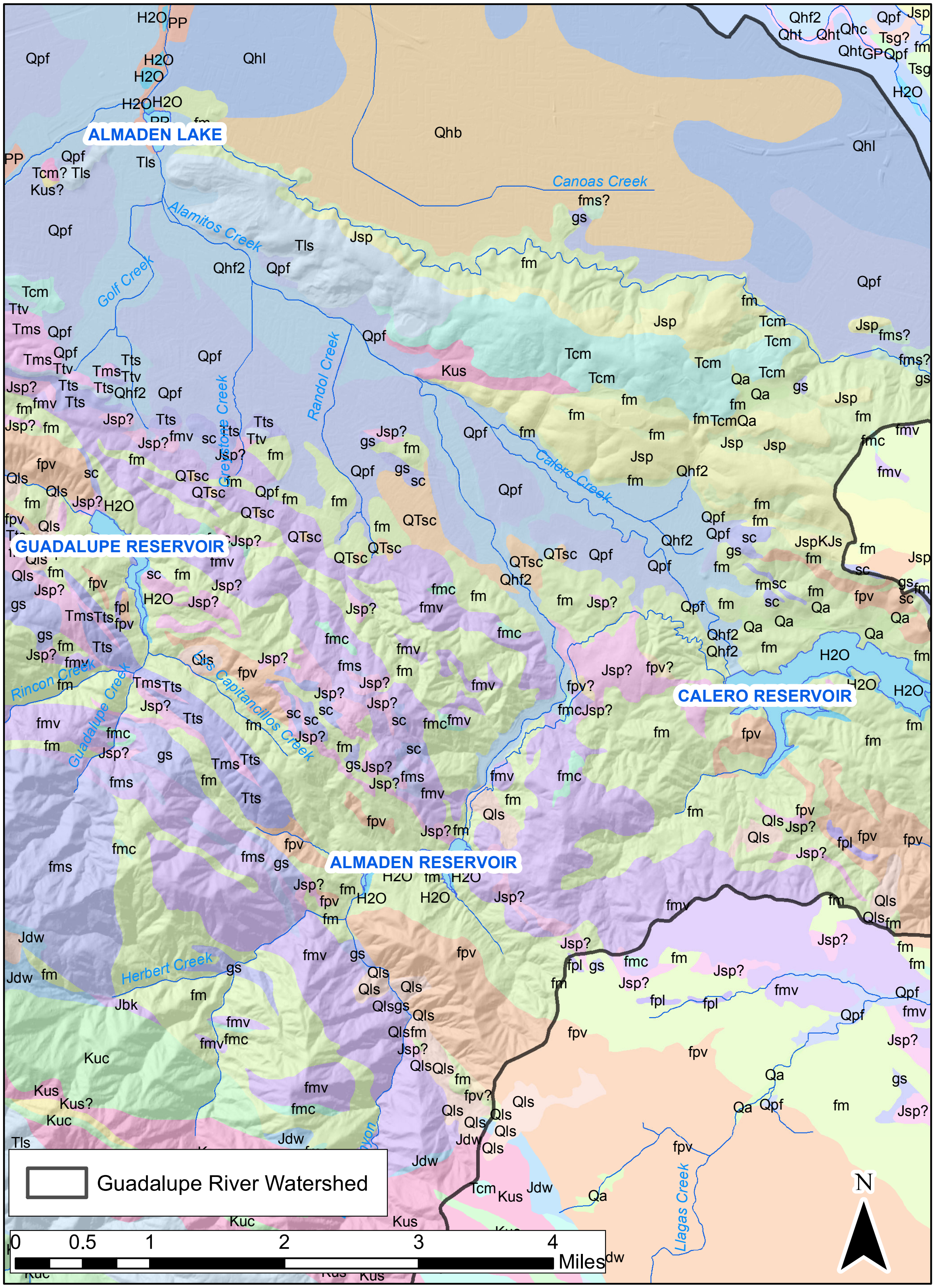
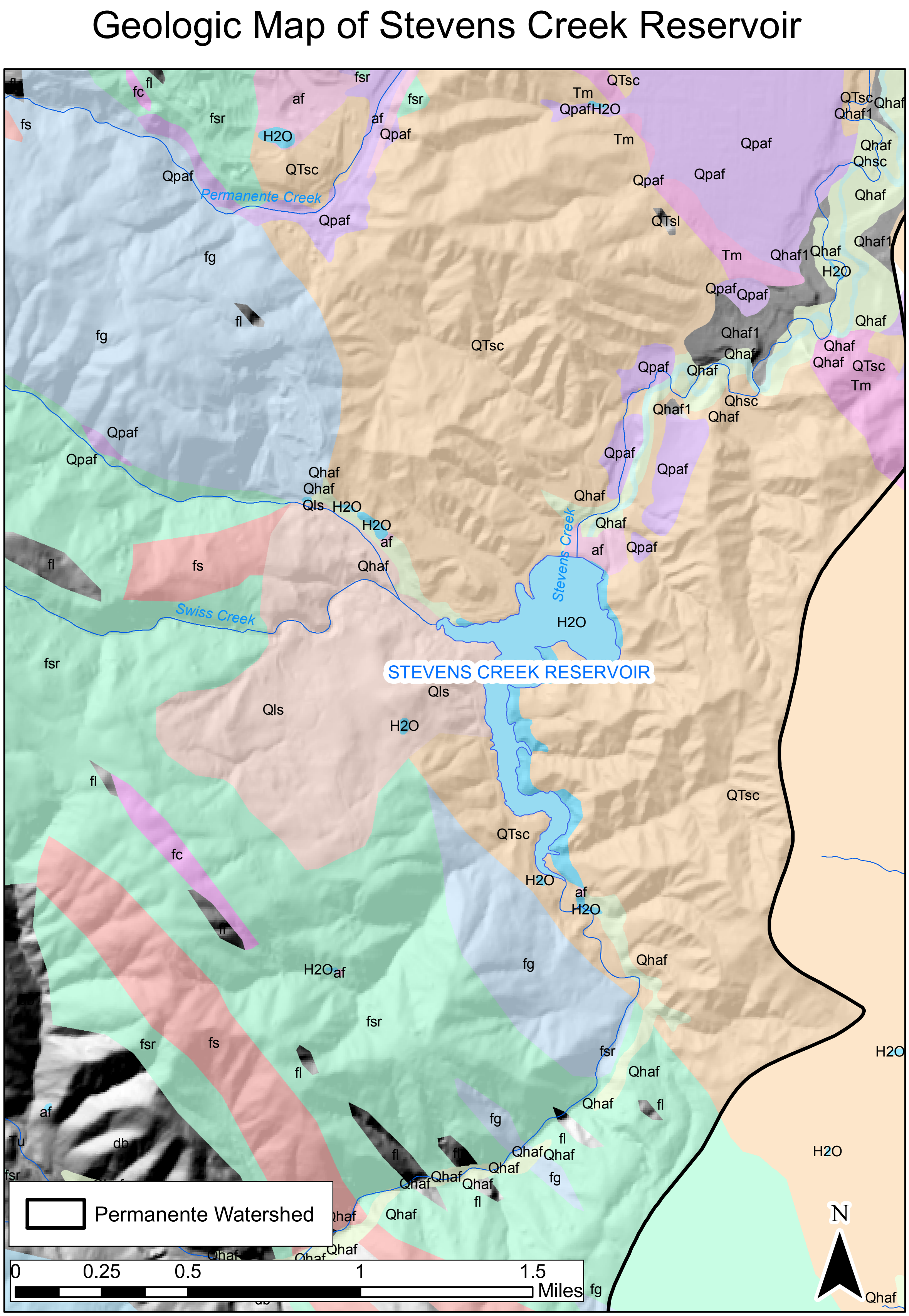


Figure A.15: Geologic Map of Stevens Creek Reservoir



B Lateral Extent of Oxygenation

Figure B.1: Almaden Reservoir

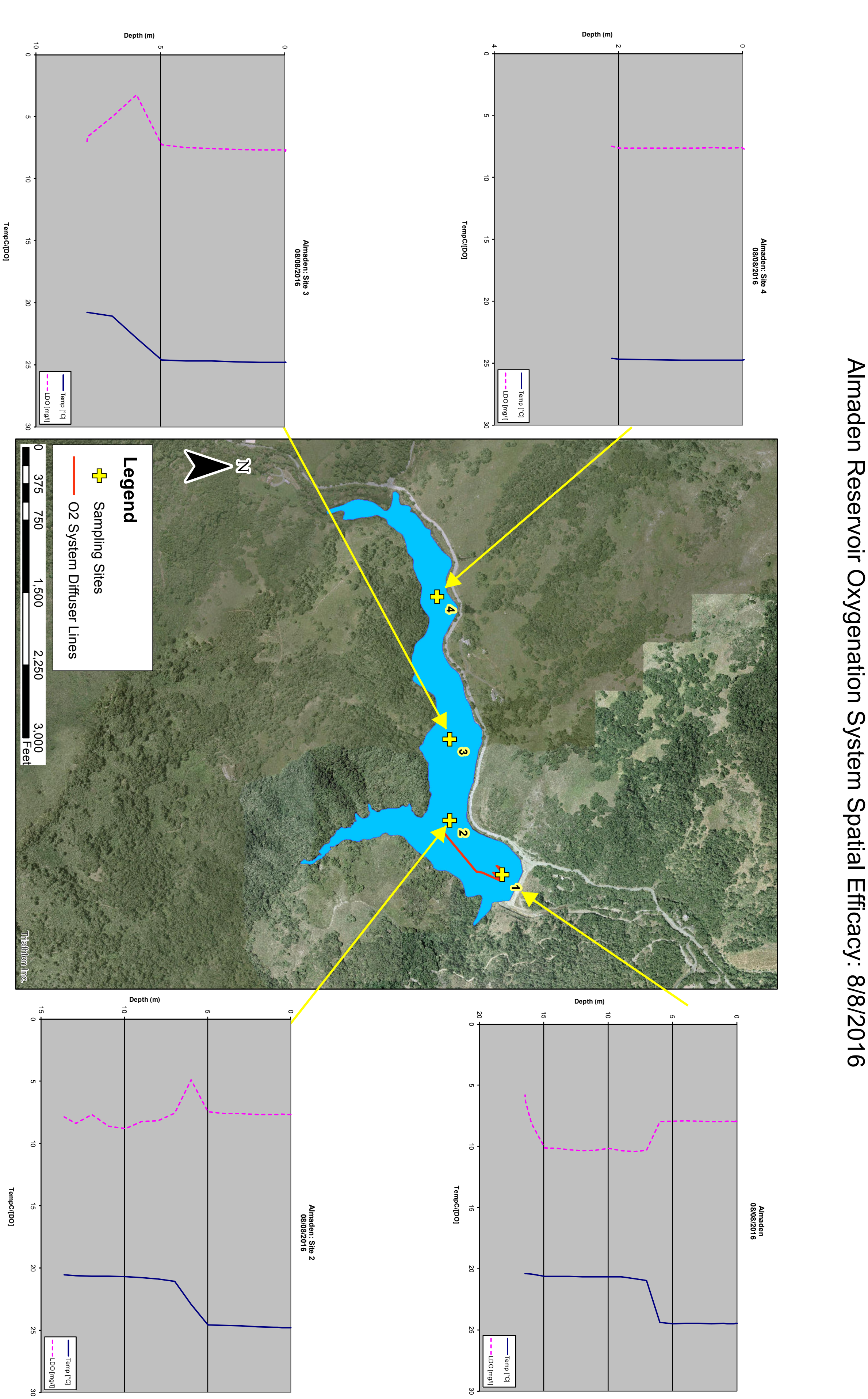


Figure B.2: Calero Reservoir

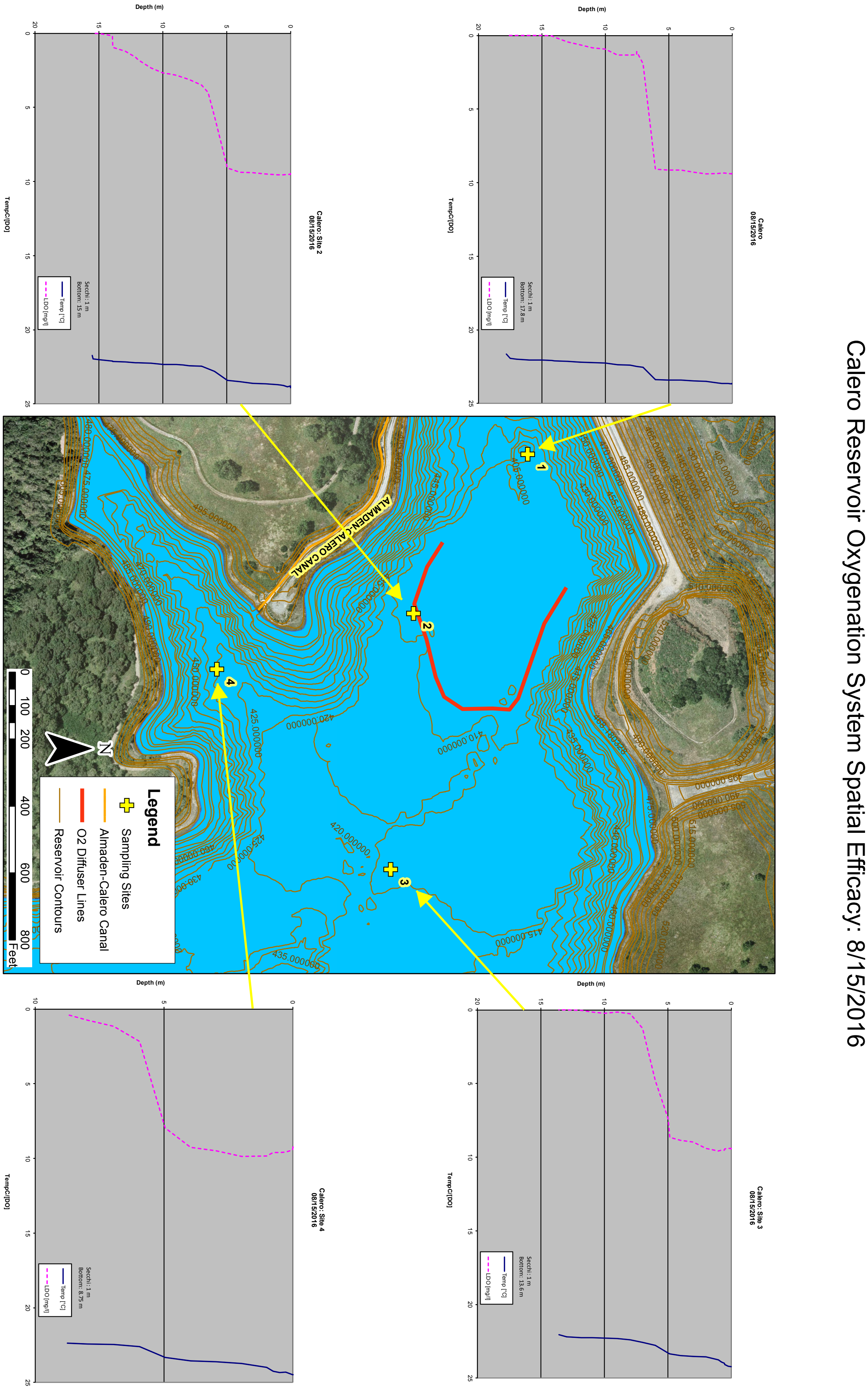
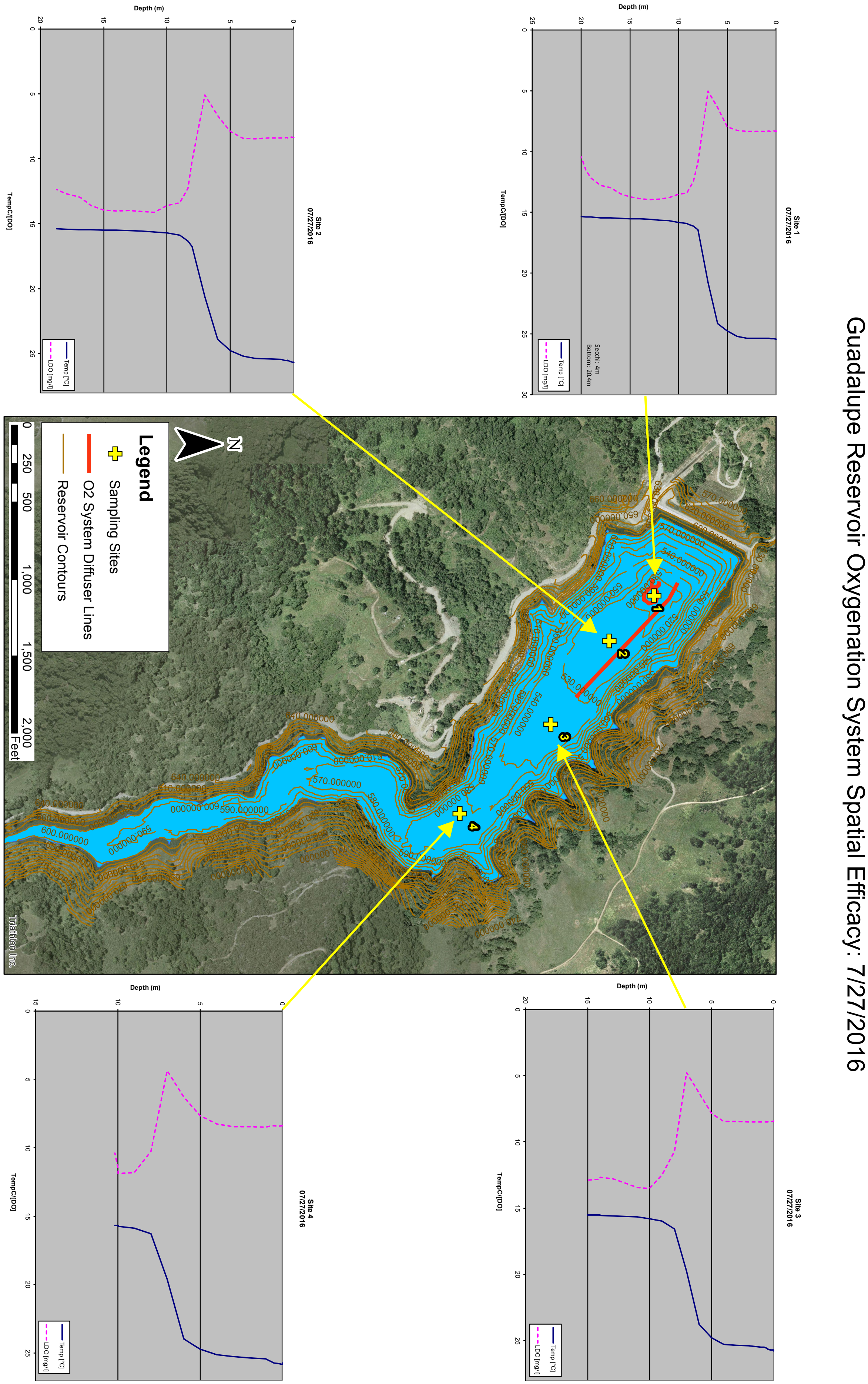
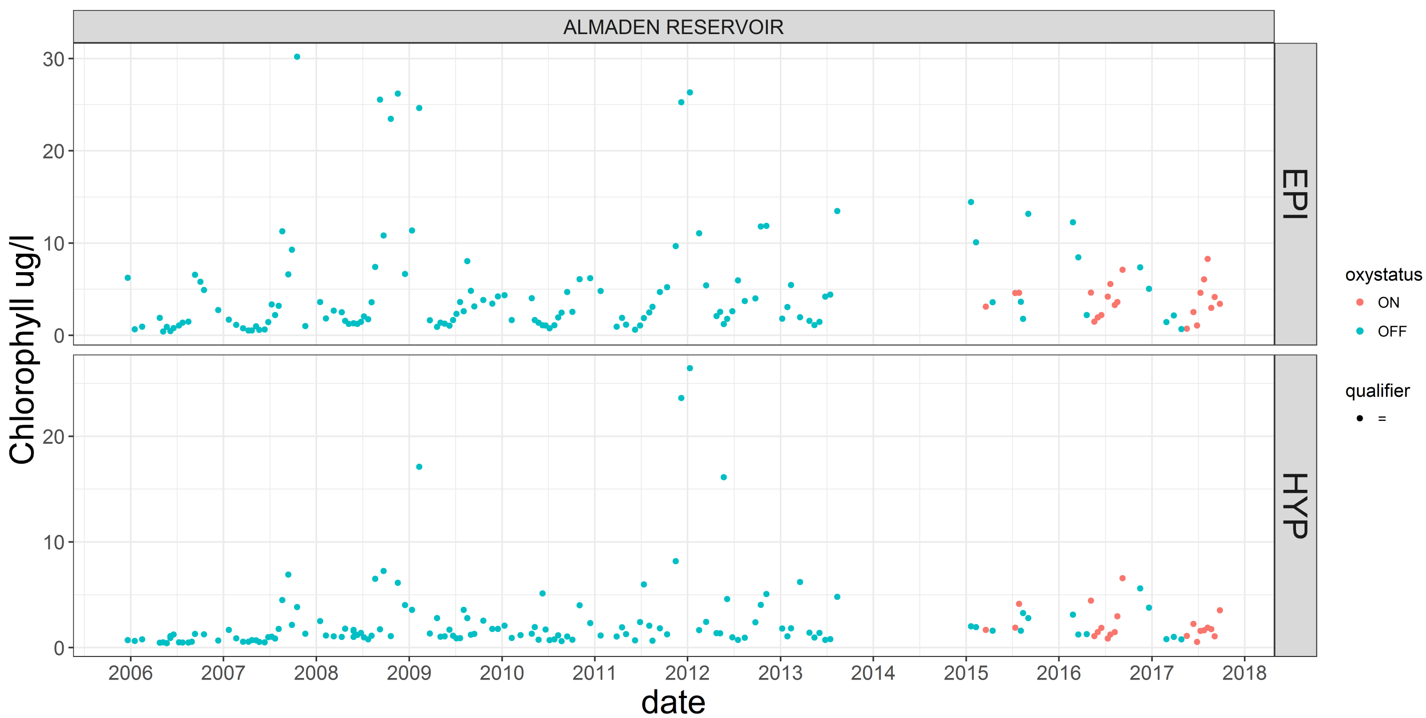


Figure B.3: Guadalupe Reservoir



C Time Series Plots


ALMADEN RESERVOIR
Ammonia mg/L
2.0
1.5
1.0
0.5
0.0
EPI
HYP
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
date
oxystatus
ON
OFF
qualifier
<
=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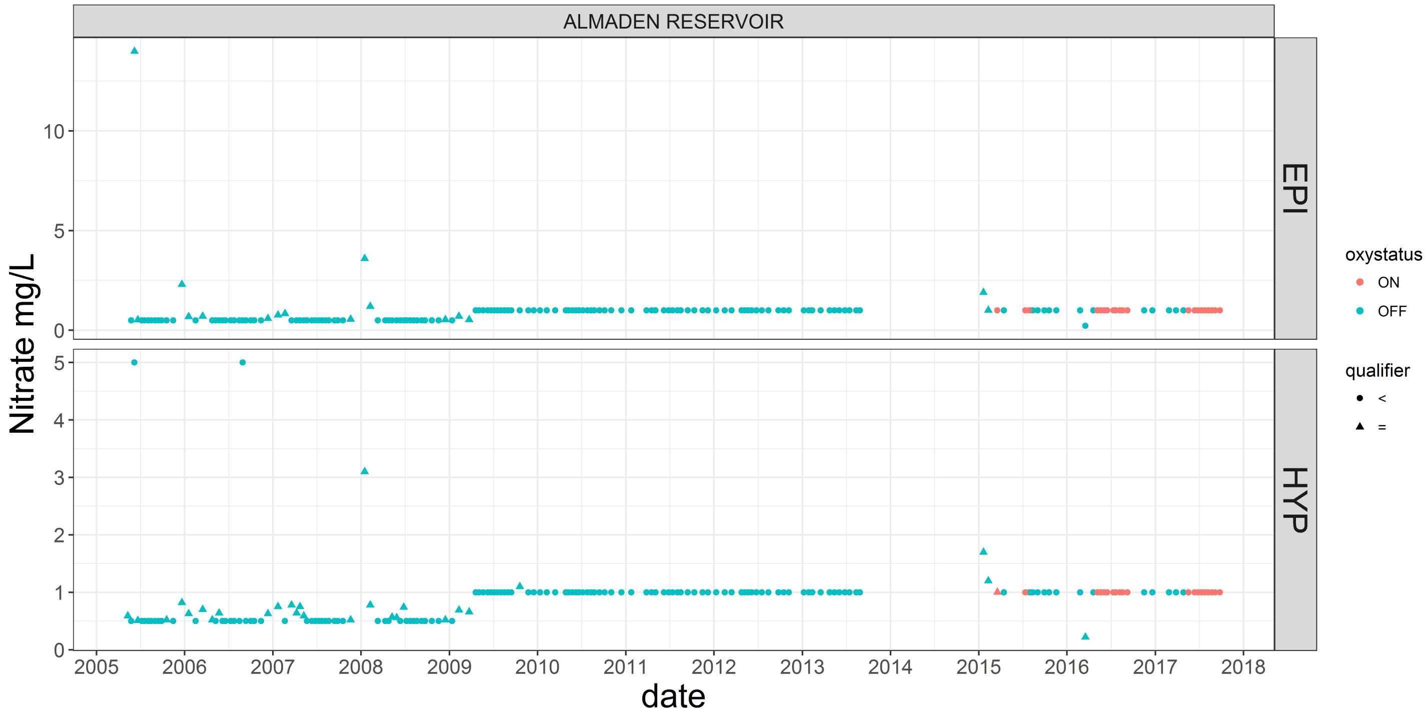
Chlorophyll ug/l
30
20
10
0
EPI
HYP
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
date
oxystatus
ON
OFF
qualifier
=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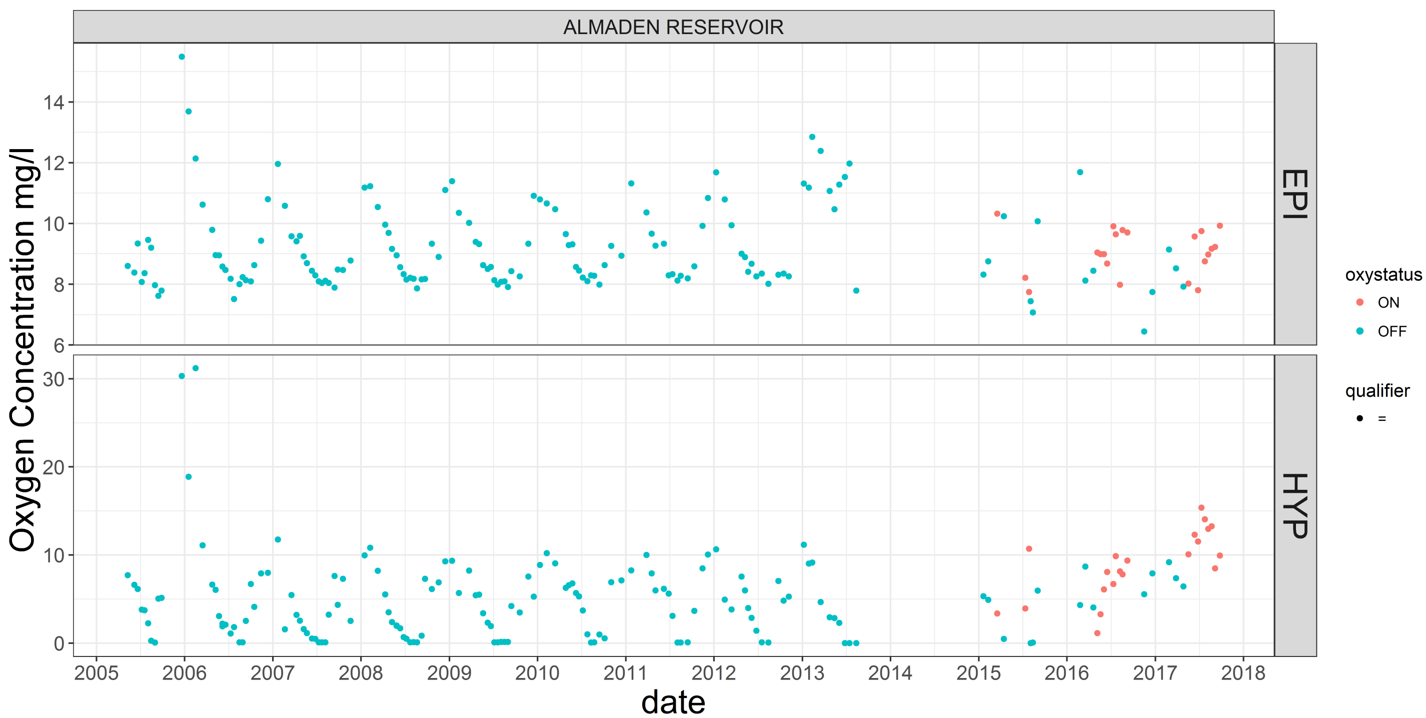
Nitrate mg/L
EPI
HYP
date
oxystatus
ON
OFF
qualifier
<
=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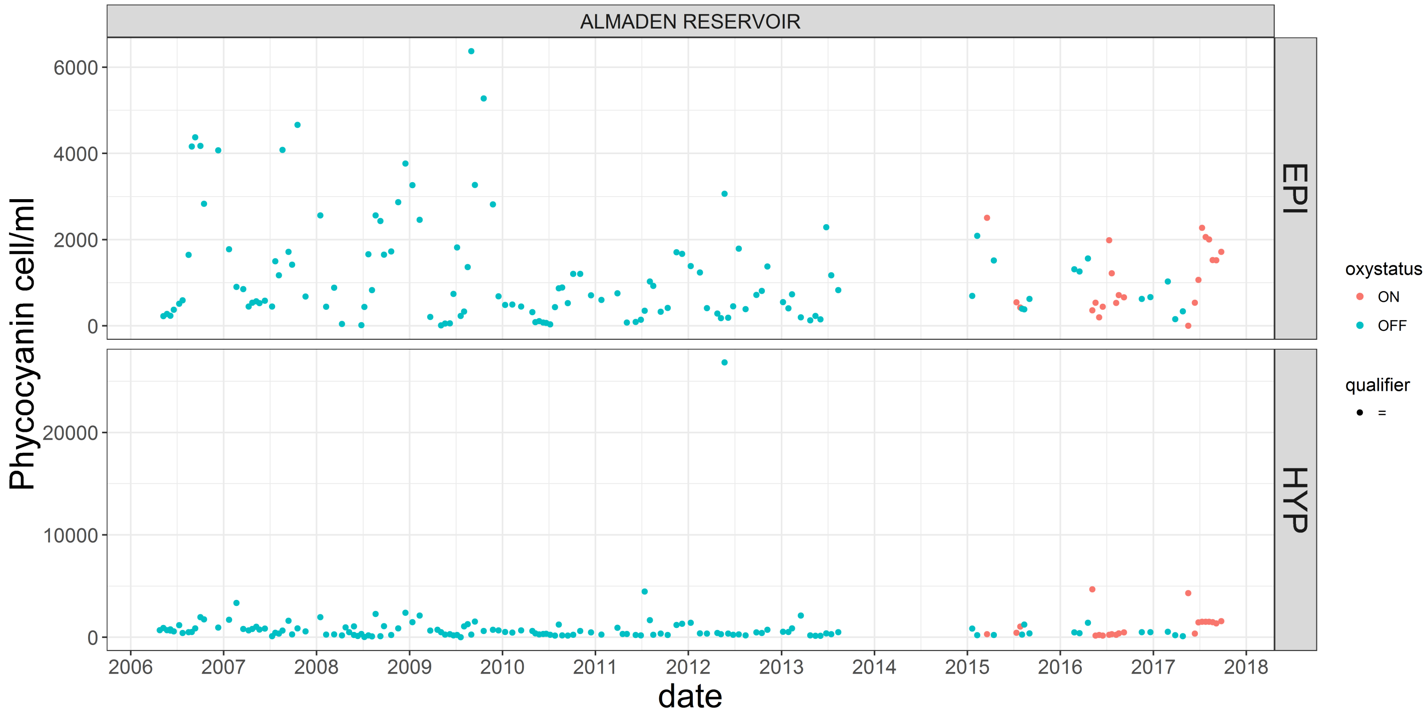
Oxygen Concentration mg/l
EPI
HYP
date
oxystatus
ON
OFF
qualifier
=



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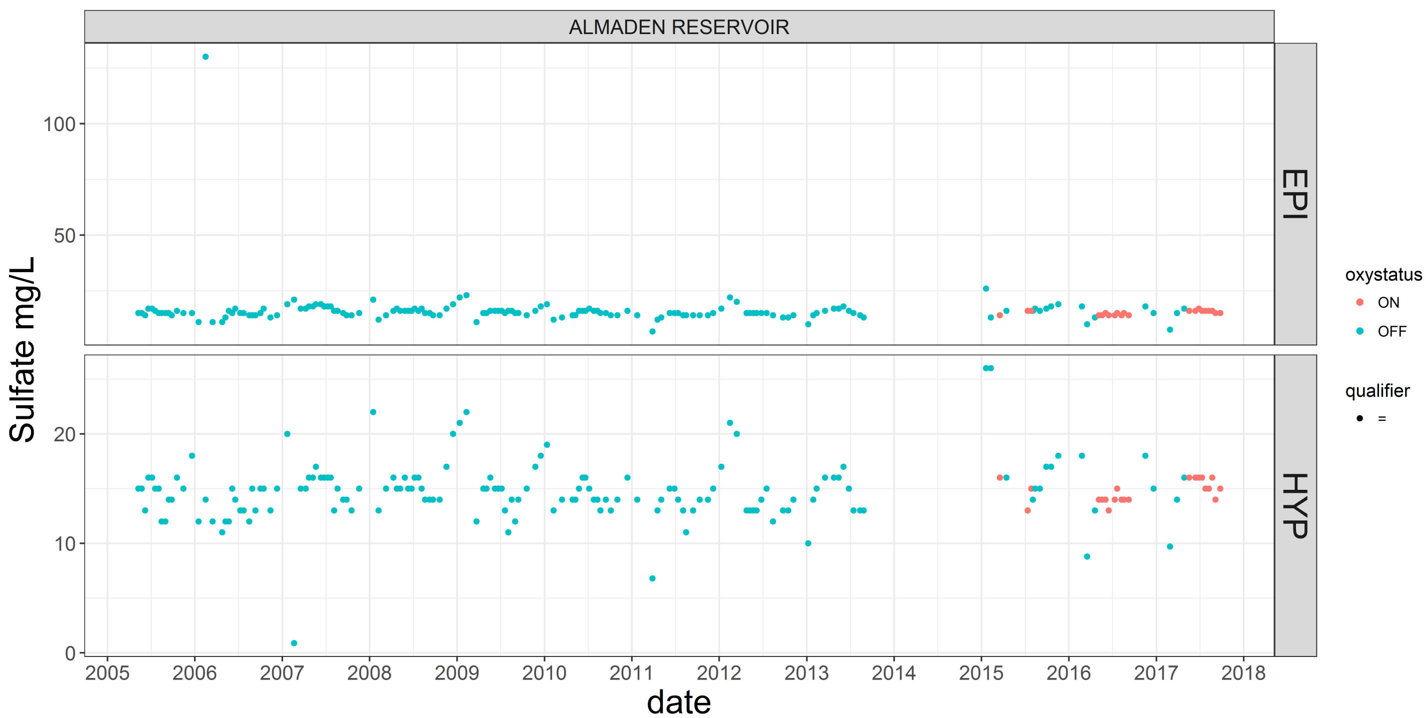
Phycocyanin cell/ml
EPI
HYP
date
oxystatus
ON
OFF
qualifier
=



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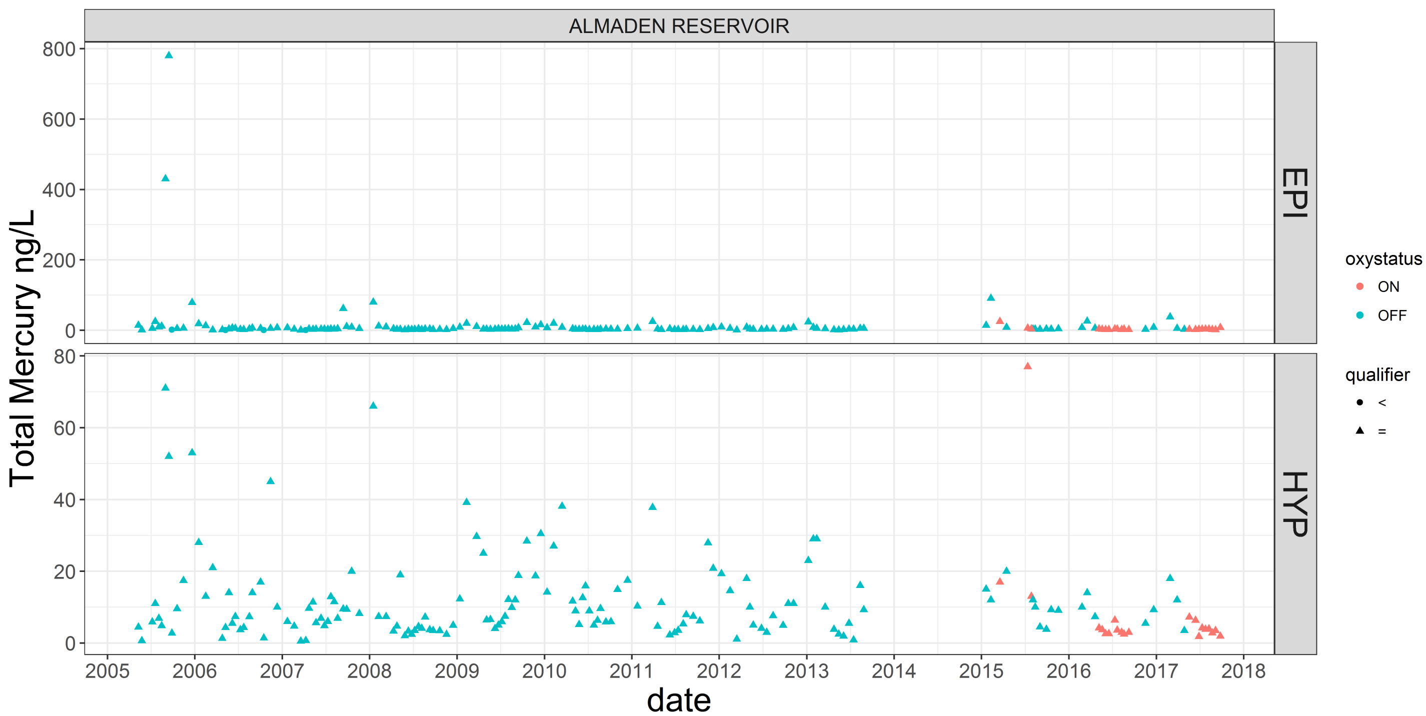
Sulfate mg/L
EPI
HYP
date
oxystatus
ON
OFF
qualifier
=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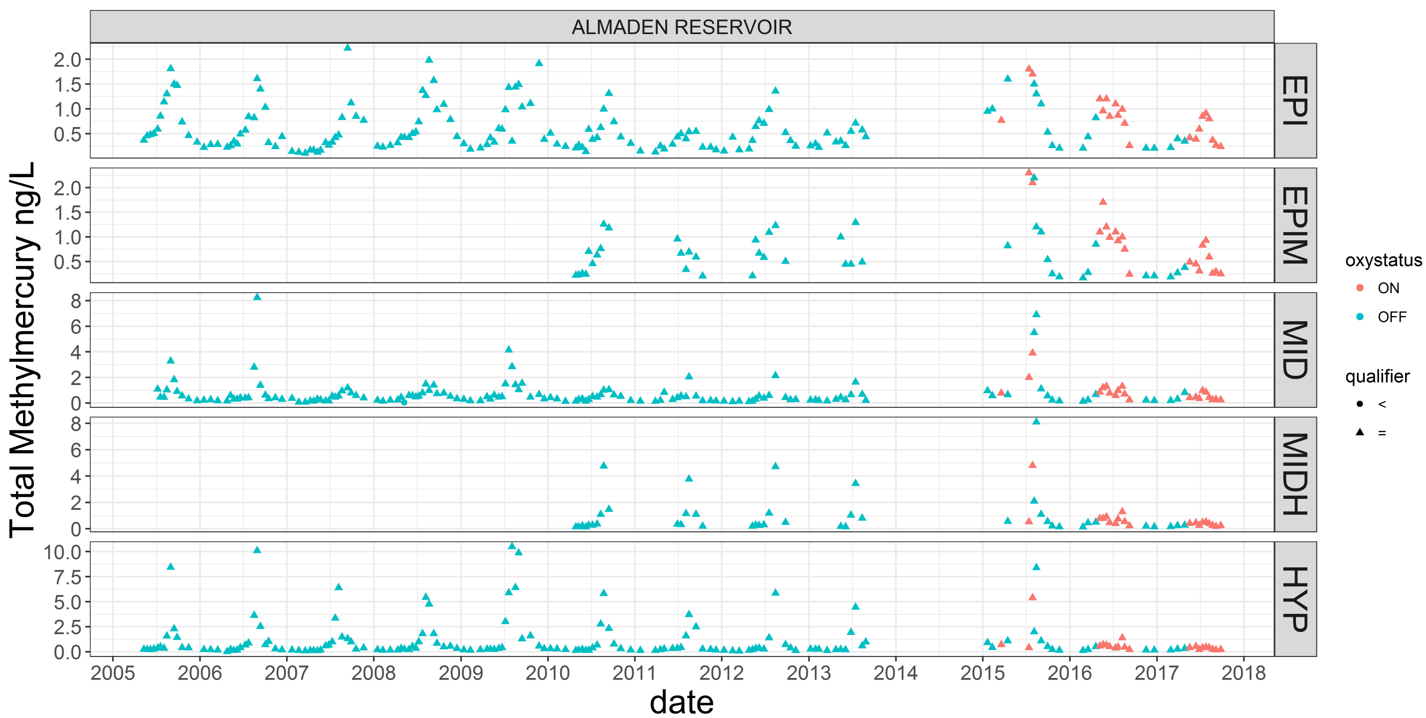
Total Mercury ng/L
EPI
HYP
date
oxystatus
ON
OFF
qualifier
<
=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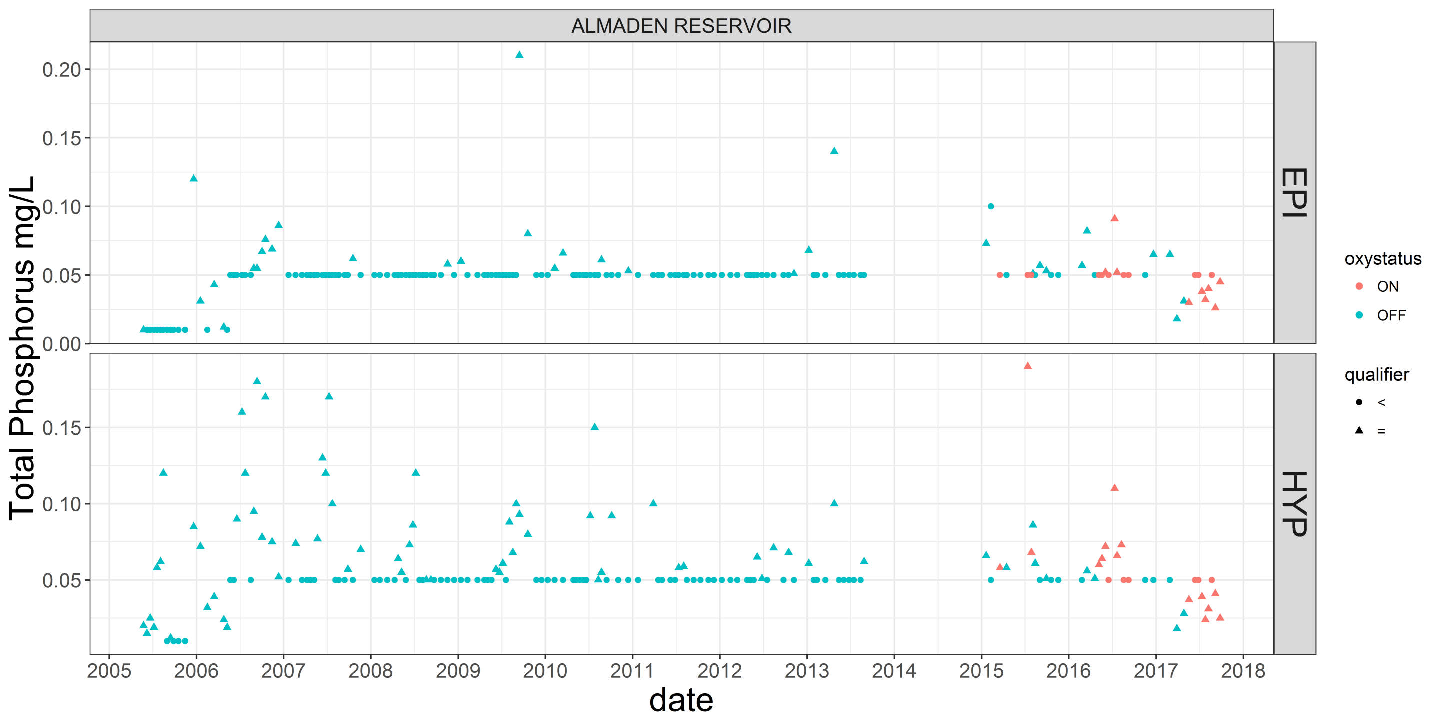
Total Methylmercury ng/L
EPI
EPIM
MID
MIDH
HYP
date
oxystatus
ON
OFF
qualifier
<
=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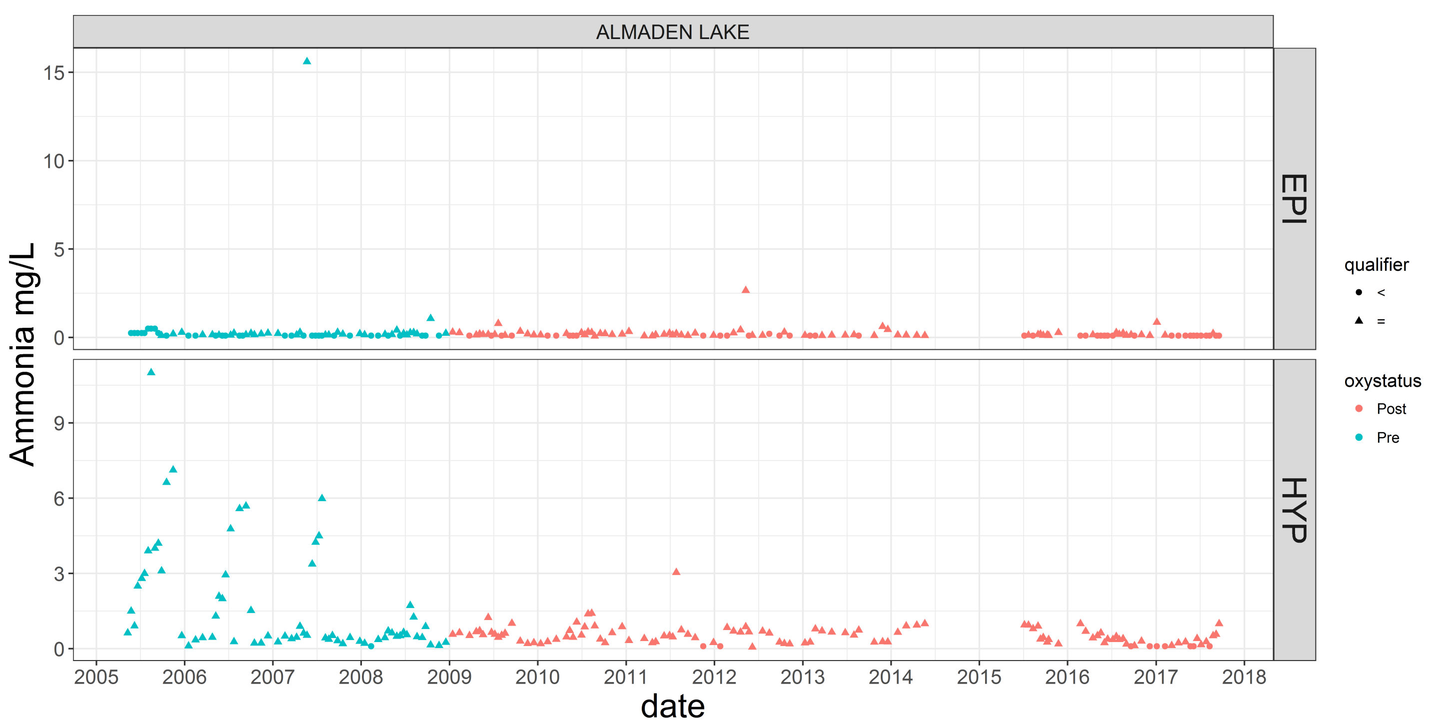
Total Phosphorus mg/L
EPI
HYP
date
oxystatus
ON
OFF
qualifier
<
=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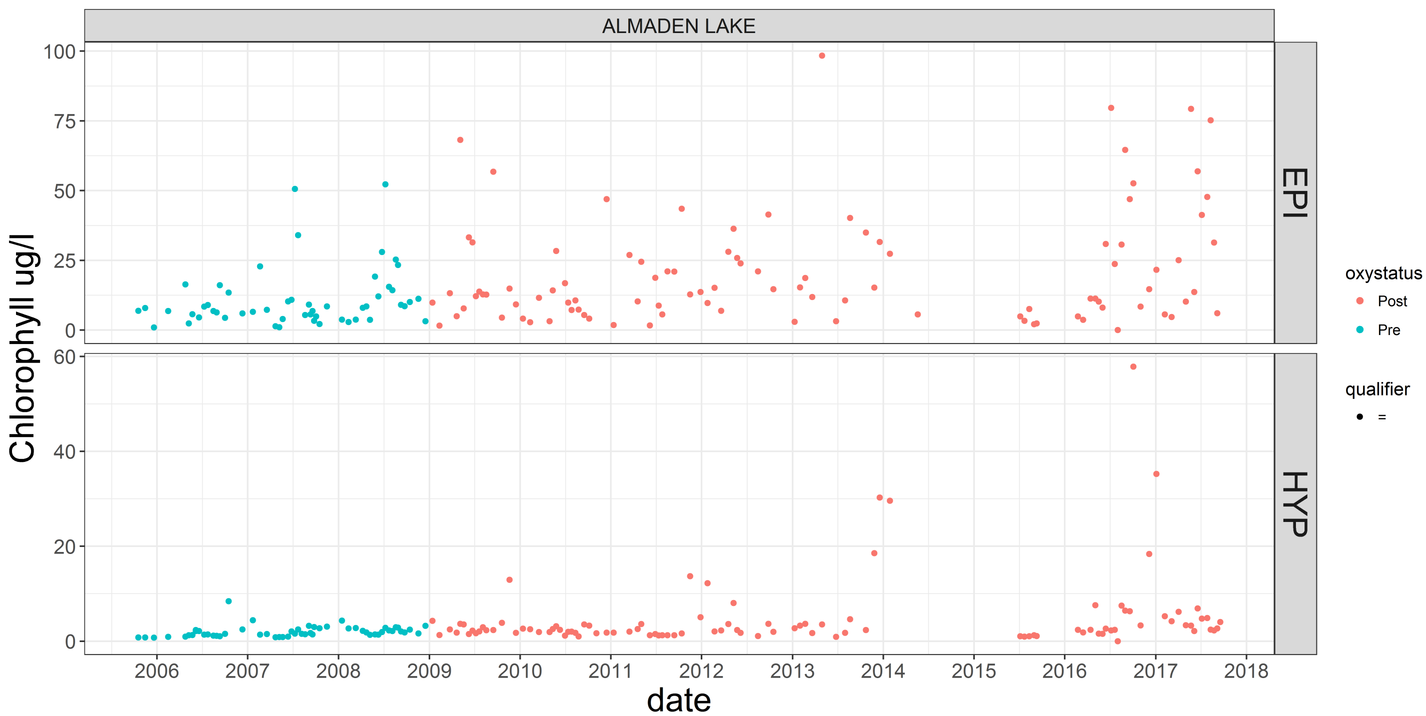
Ammonia mg/L
EPI
HYP
date
qualifier
<
=
oxystatus
Post
Pre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Chlorophyll ug/l
EPI
HYP
date
oxystatus
Post
Pre
qualifier
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60
40
20
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2007
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2011
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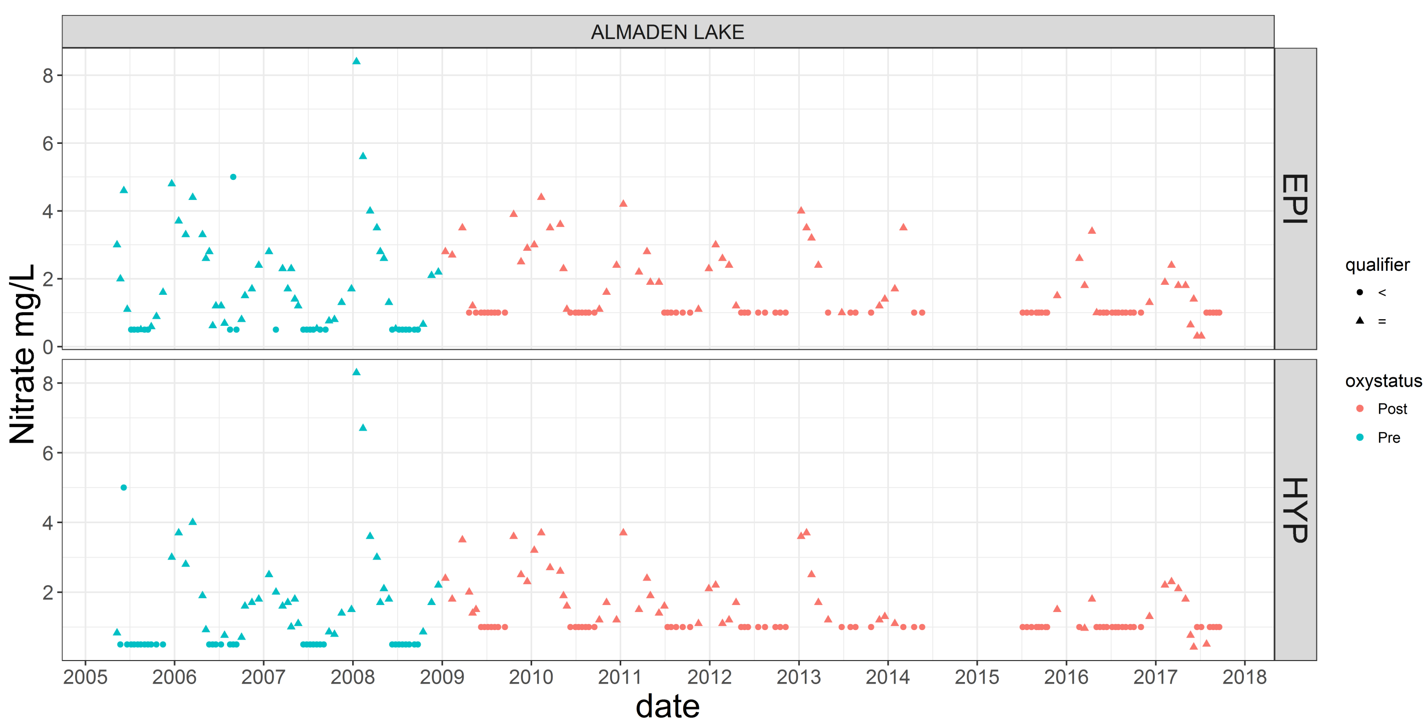
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Nitrate mg/L
EPI
HYP
date
qualifier
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oxystatus
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Pre
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2005
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2007
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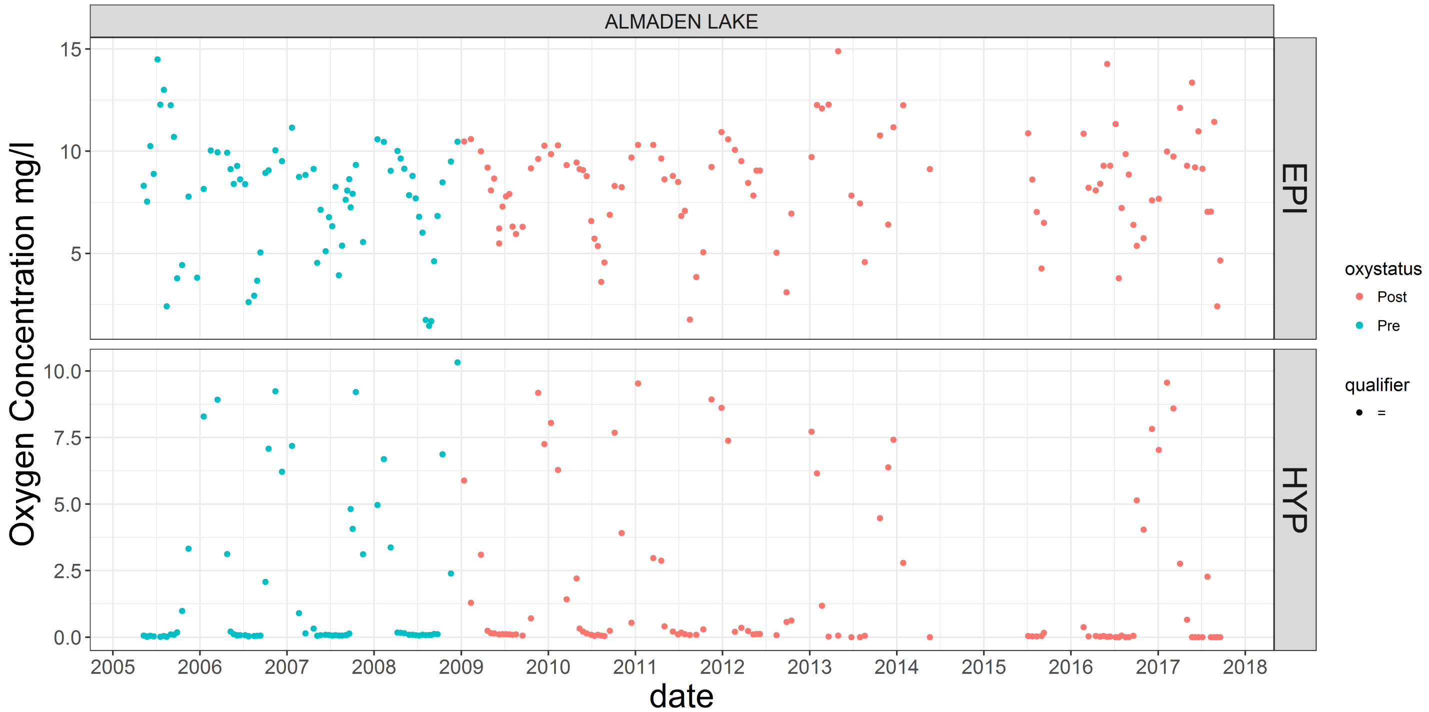
2018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HYP
date
oxystatus
Post
Pre
qualifier
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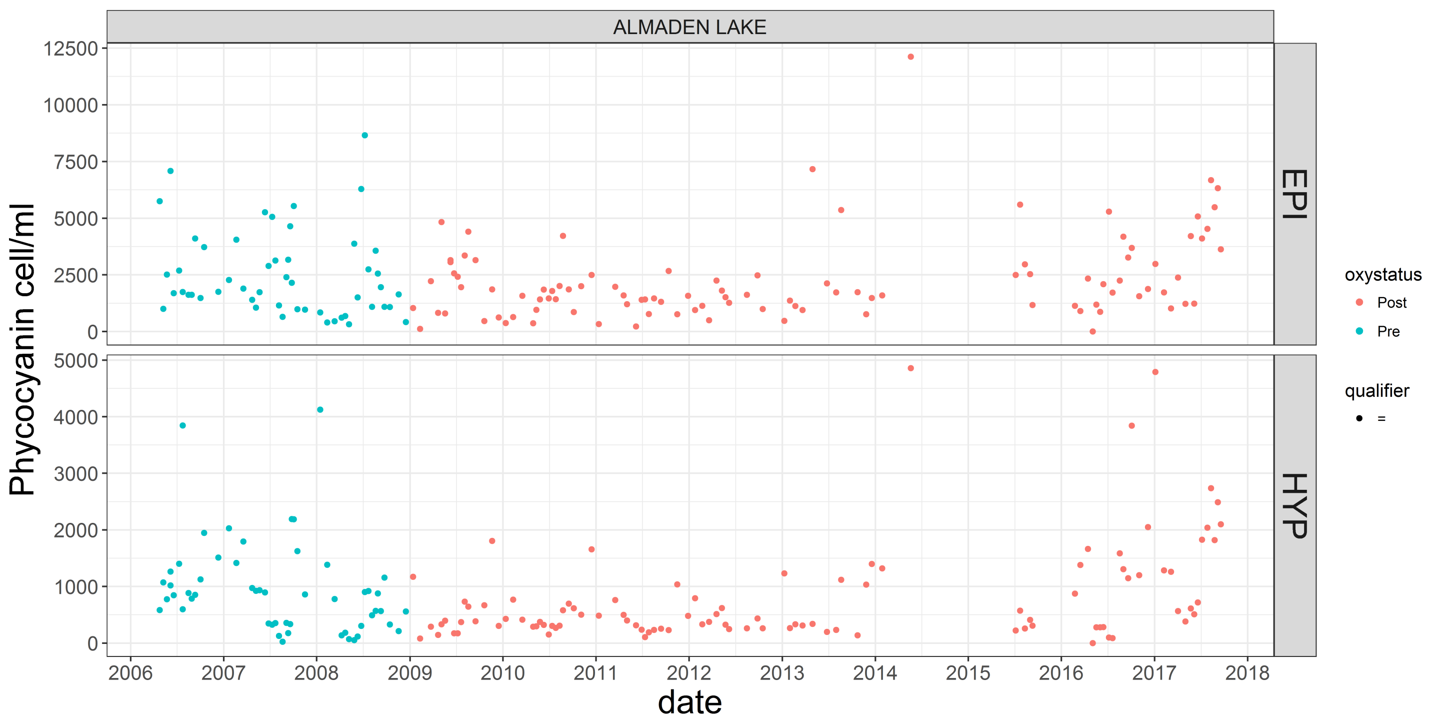
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EPI
HYP
date
oxystatus
Post
Pre
qualifier
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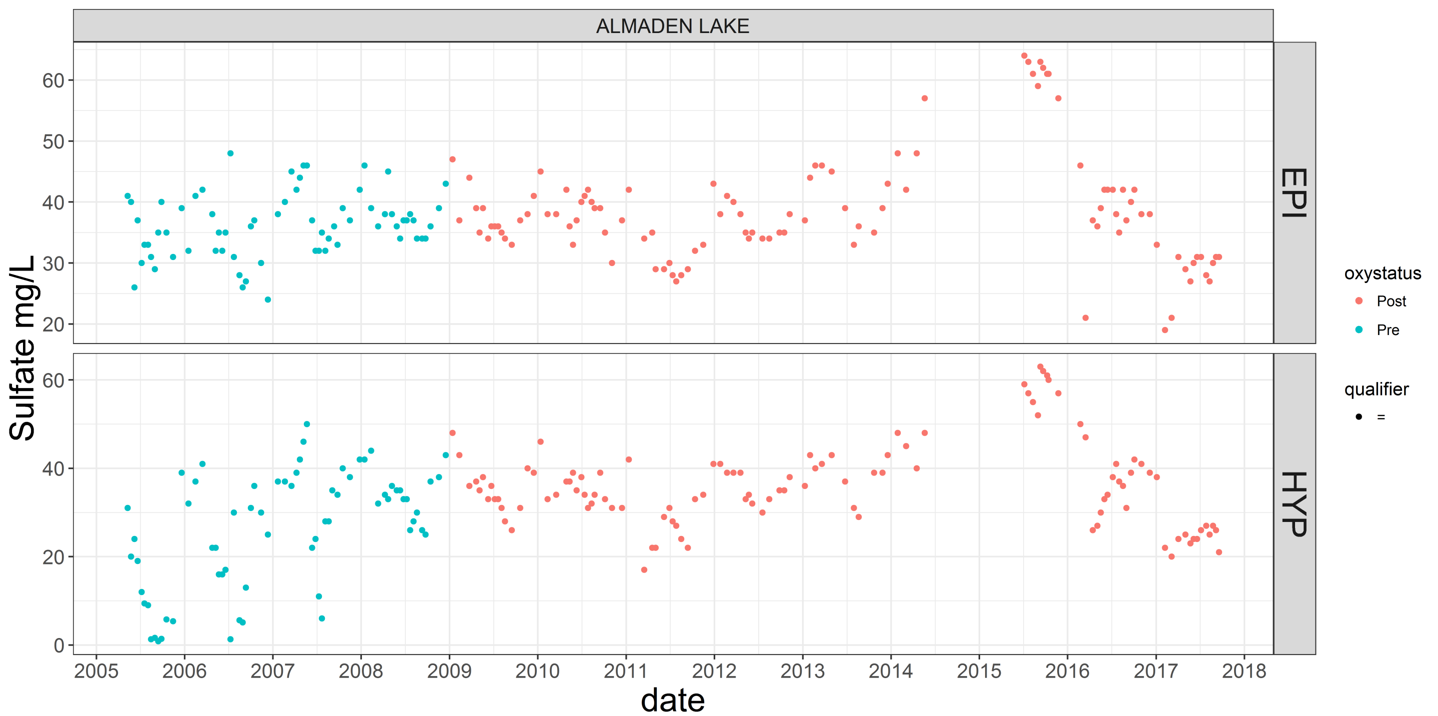
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Sulfate mg/L
EPI
HYP
date
oxystatus
Post
Pre
qualifier
=
2005
2006
2007
2008
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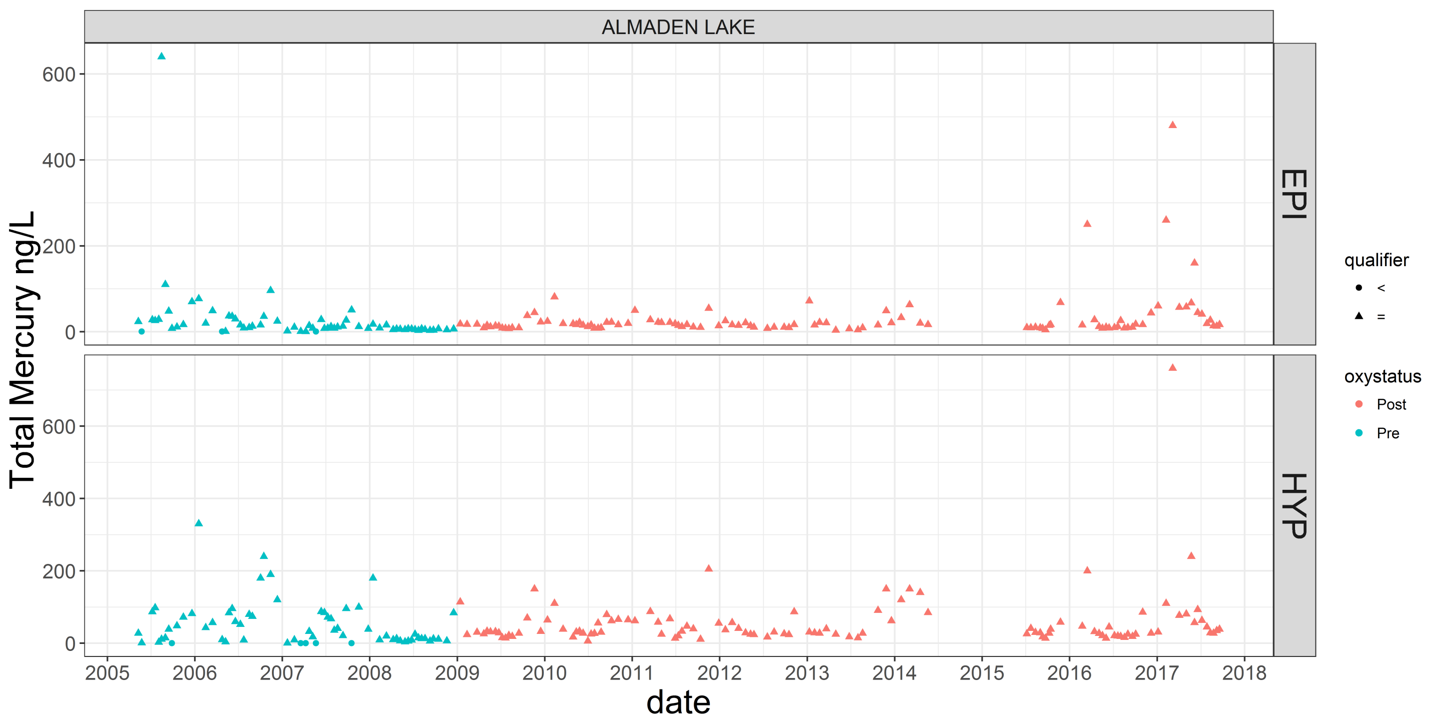
2018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HYP
date
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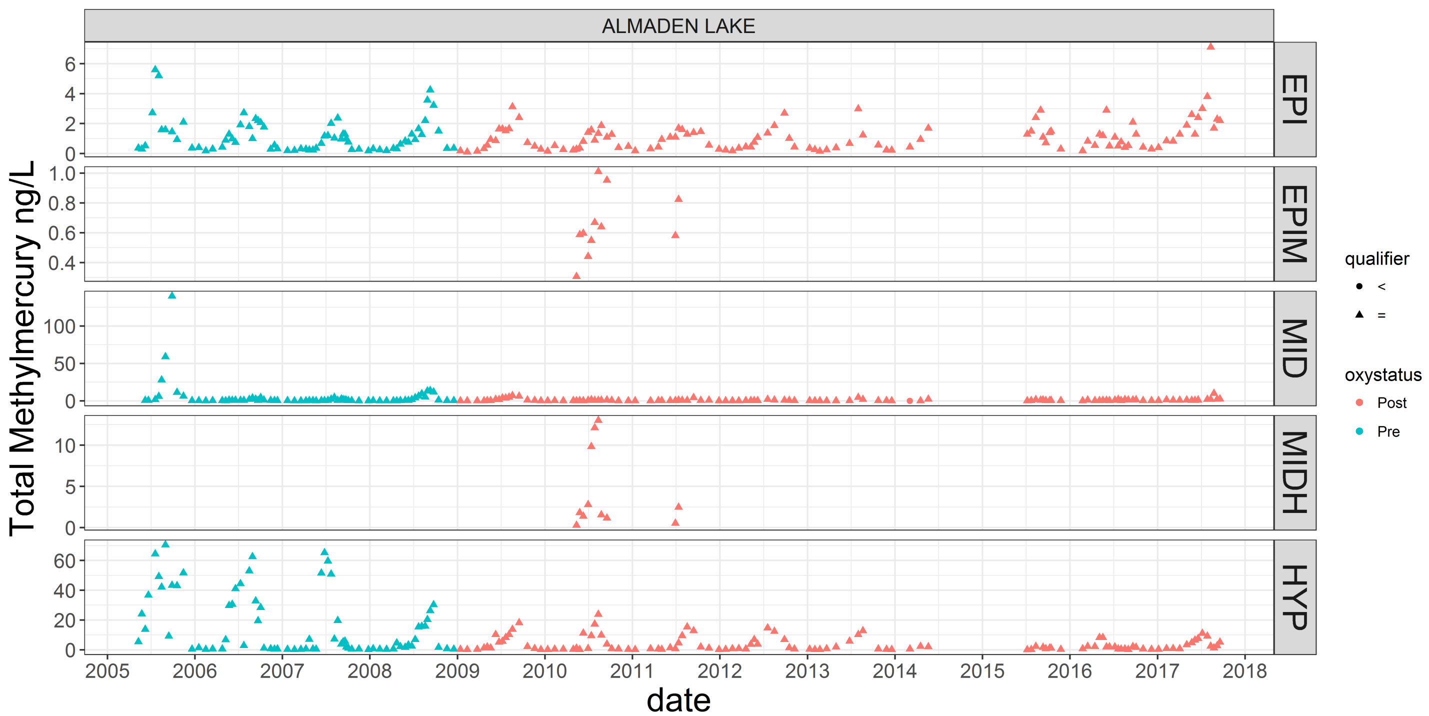
=
oxystatus
Post
Pre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MID
MIDH
HYP
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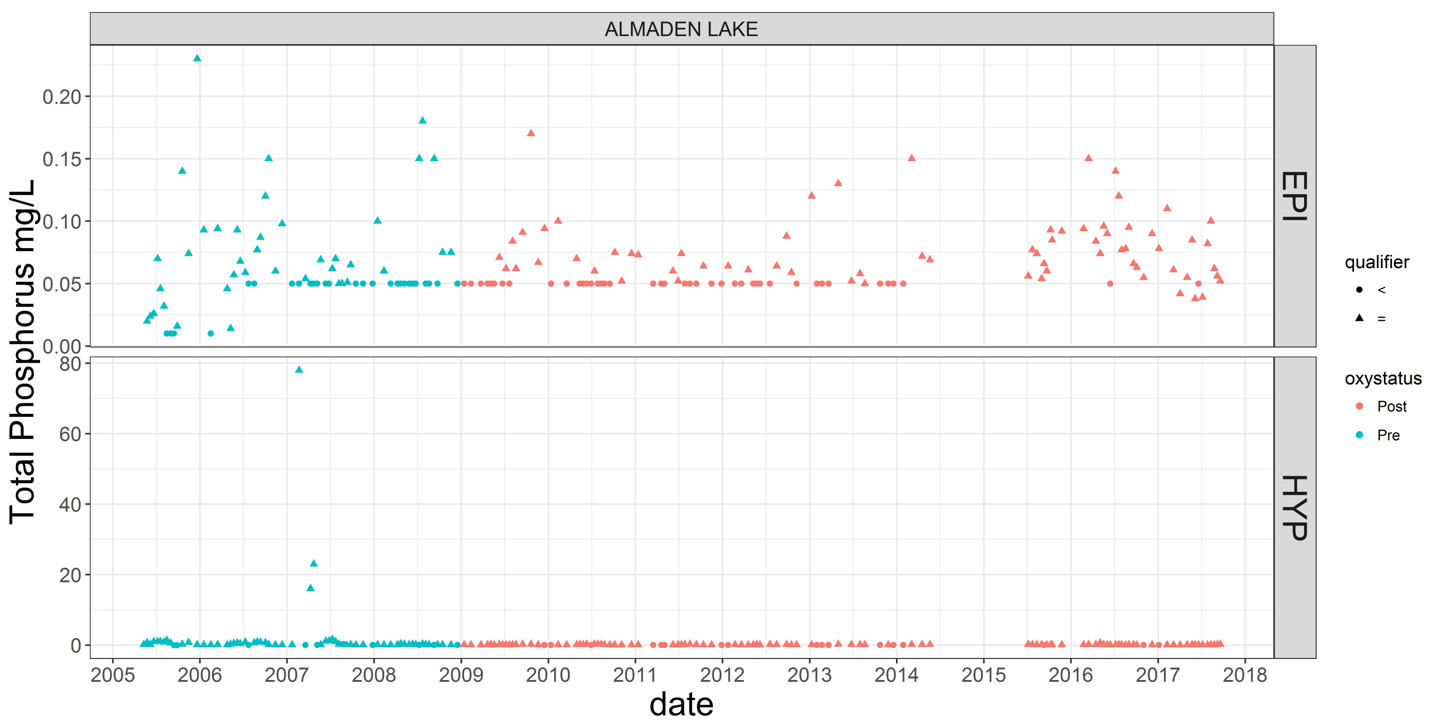
oxystatus
Post
Pre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HYP
date
qualifier
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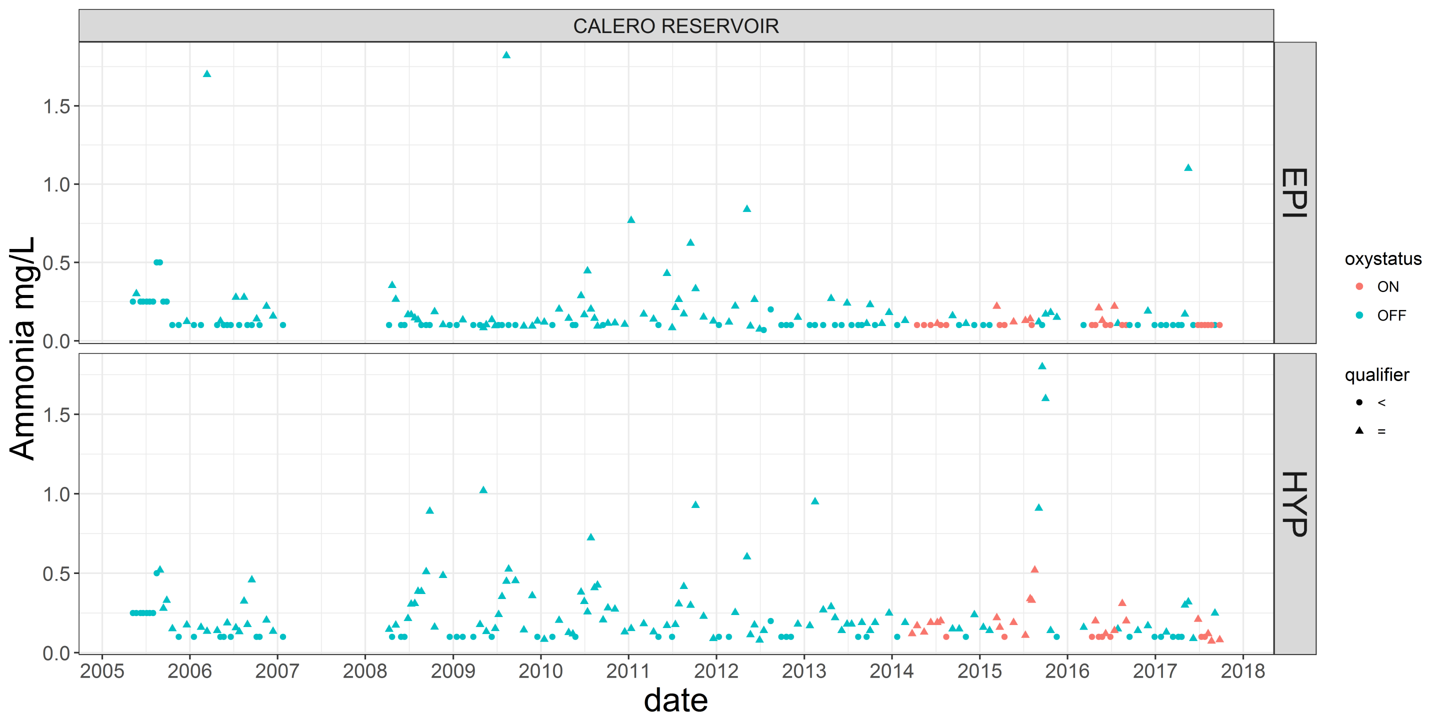
=
oxystatus
Post
Pre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Ammonia mg/L
EPI
HYP
date
oxystatus
ON
OFF
qualifier
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=
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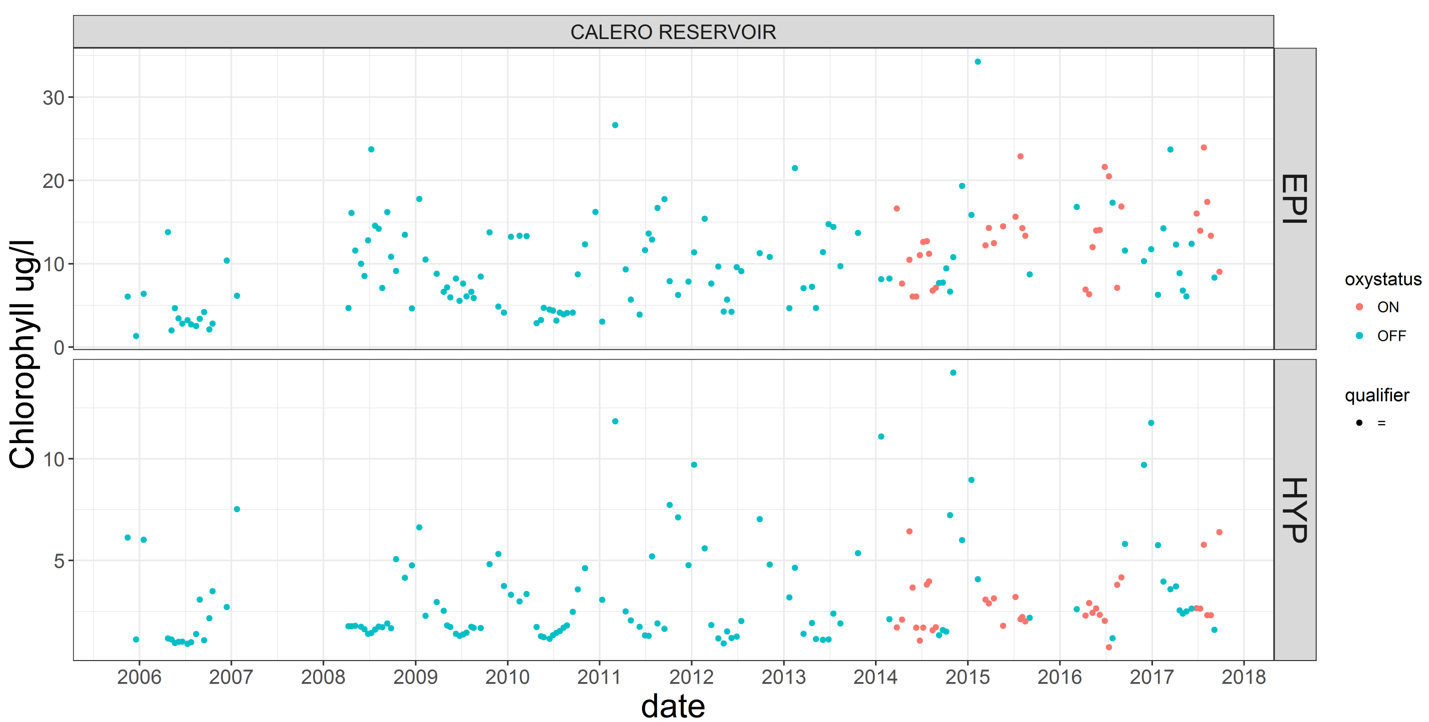
2015
2016
2017
2018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Chlorophyll ug/l
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HYP
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oxystatus
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OFF
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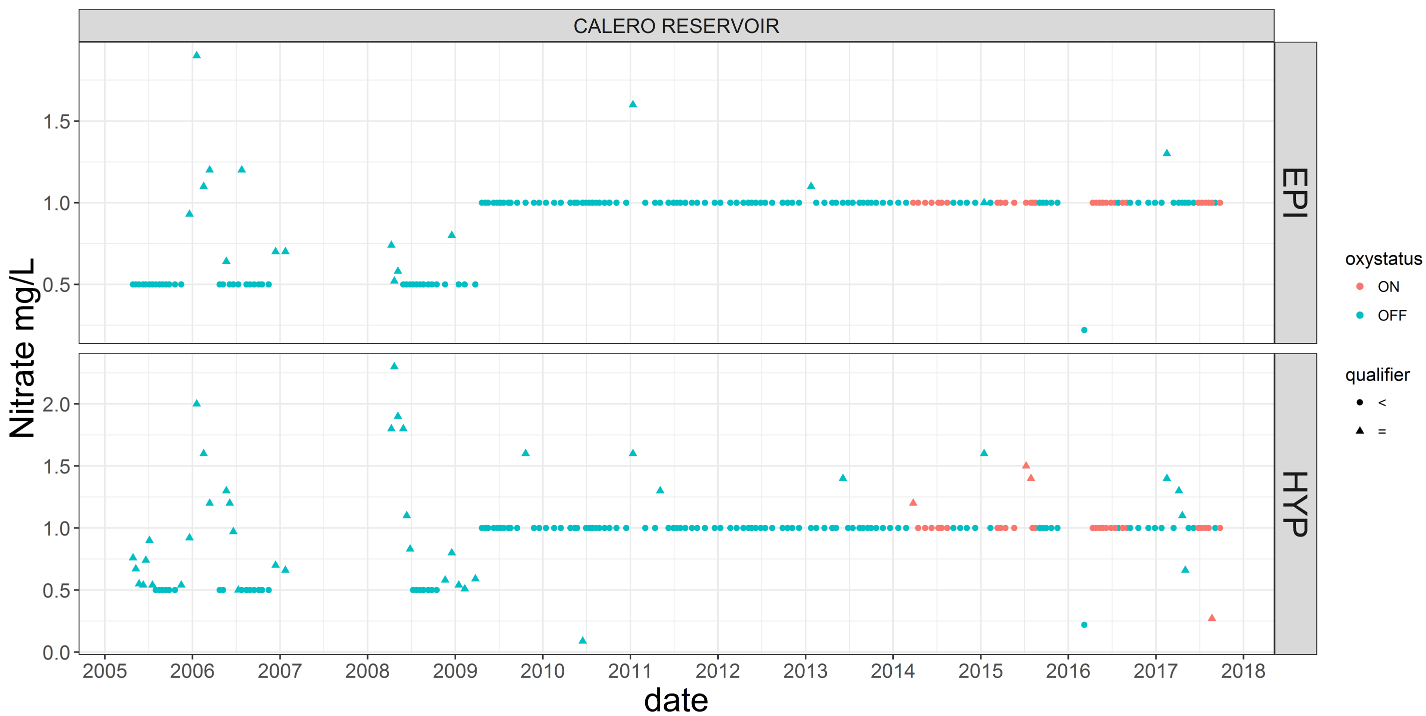
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oxystatus
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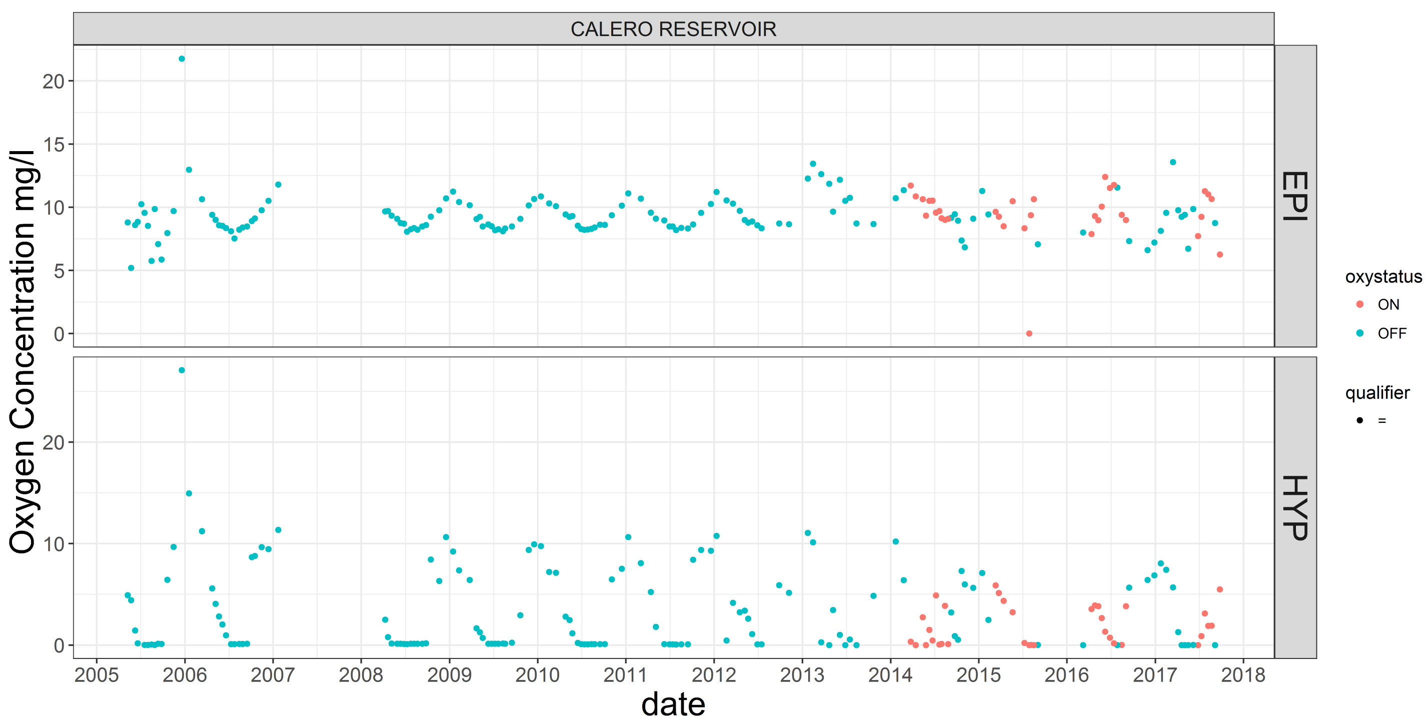
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HYP
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qualifier
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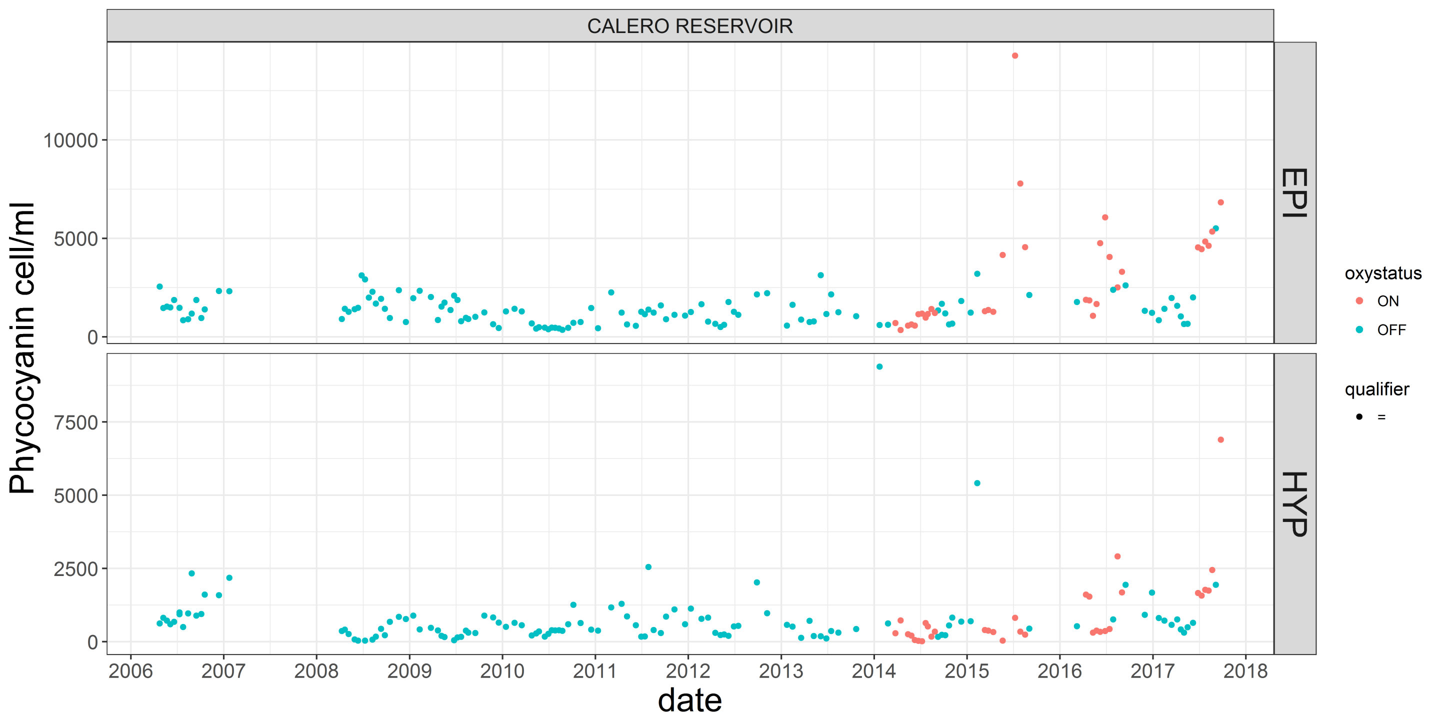
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Phycocyanin cell/ml
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0
7500
5000
2500
0
EPI
HYP
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2007
2008
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2010
2011
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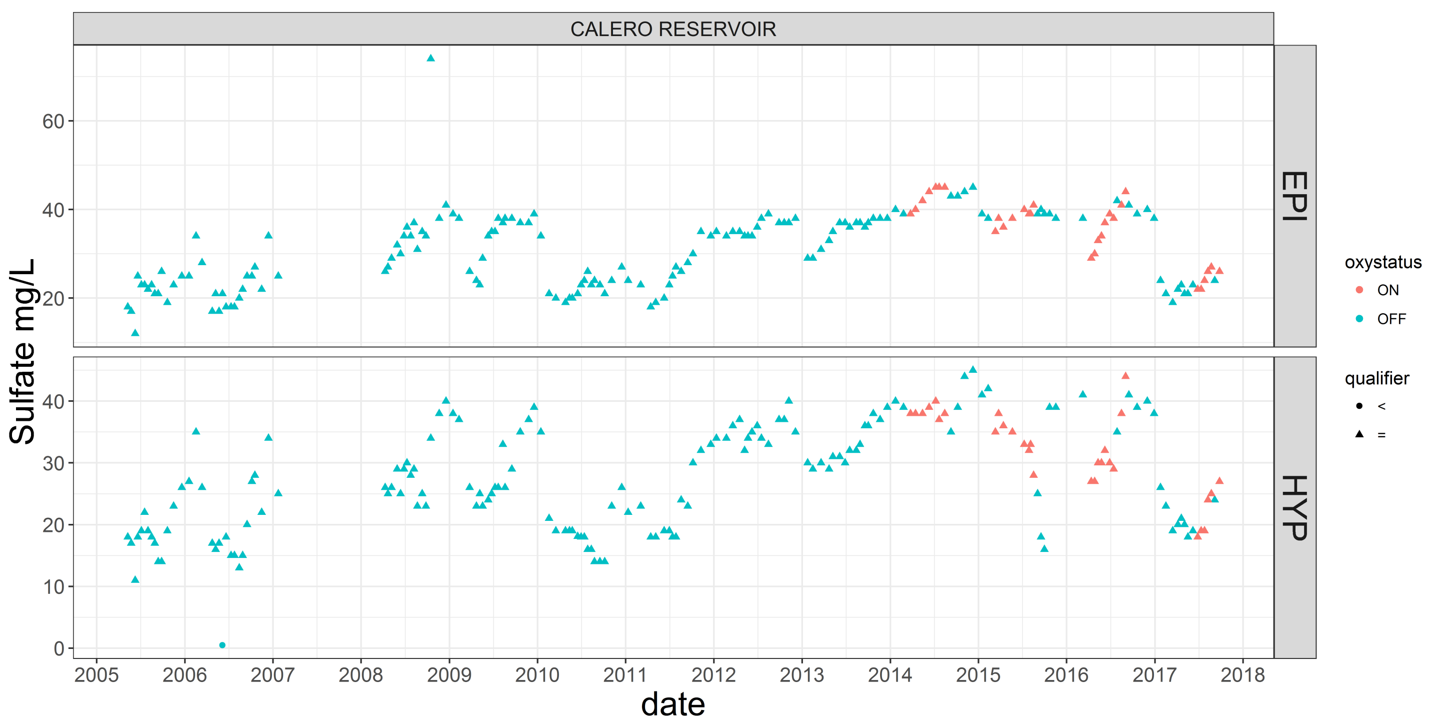
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40
20
30
10
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EPI
HYP
2005
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date
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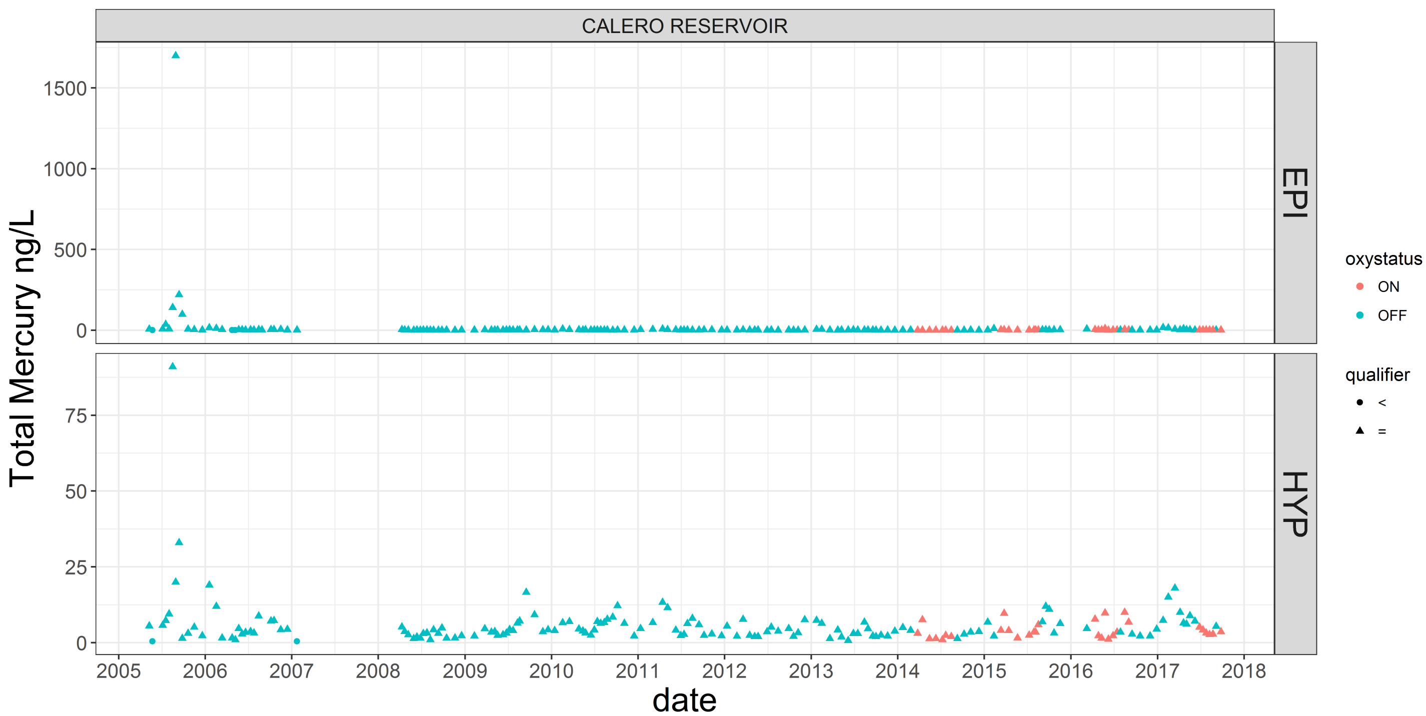
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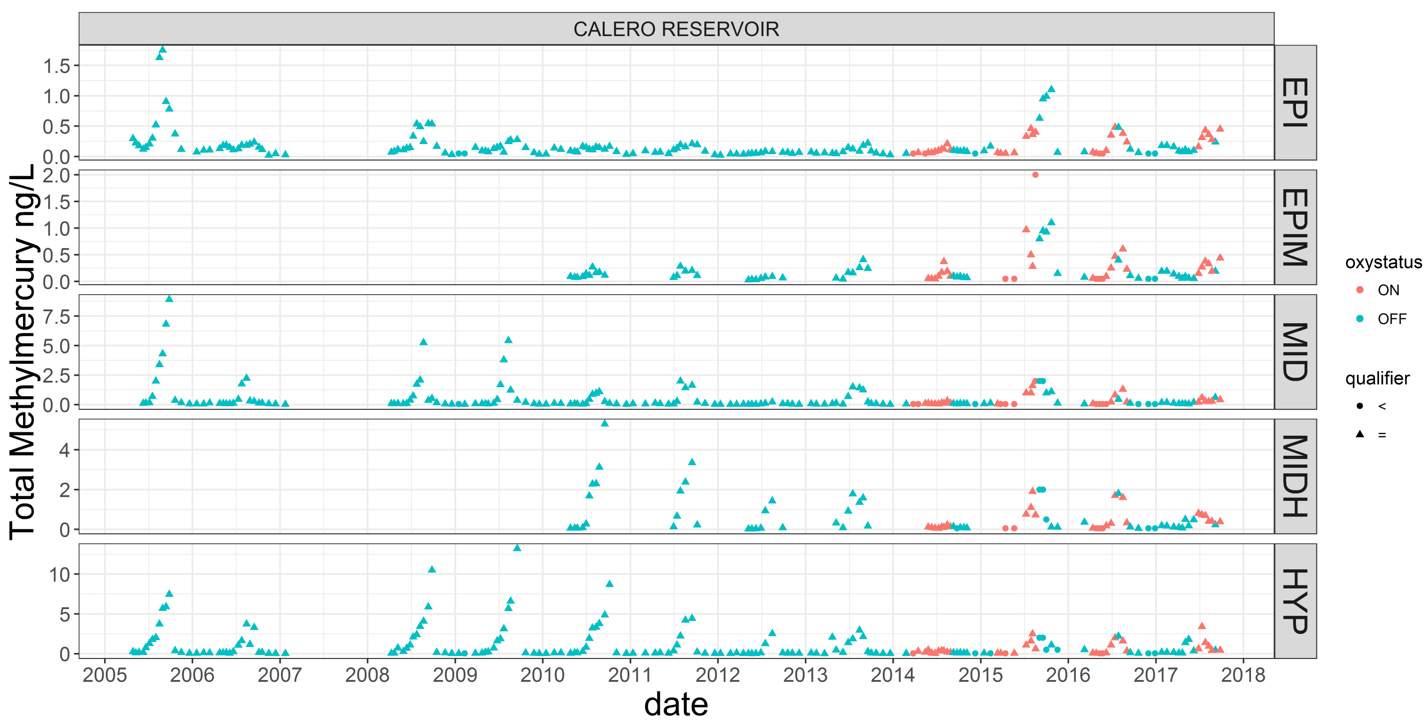
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EPIM
MID
MIDH
HYP
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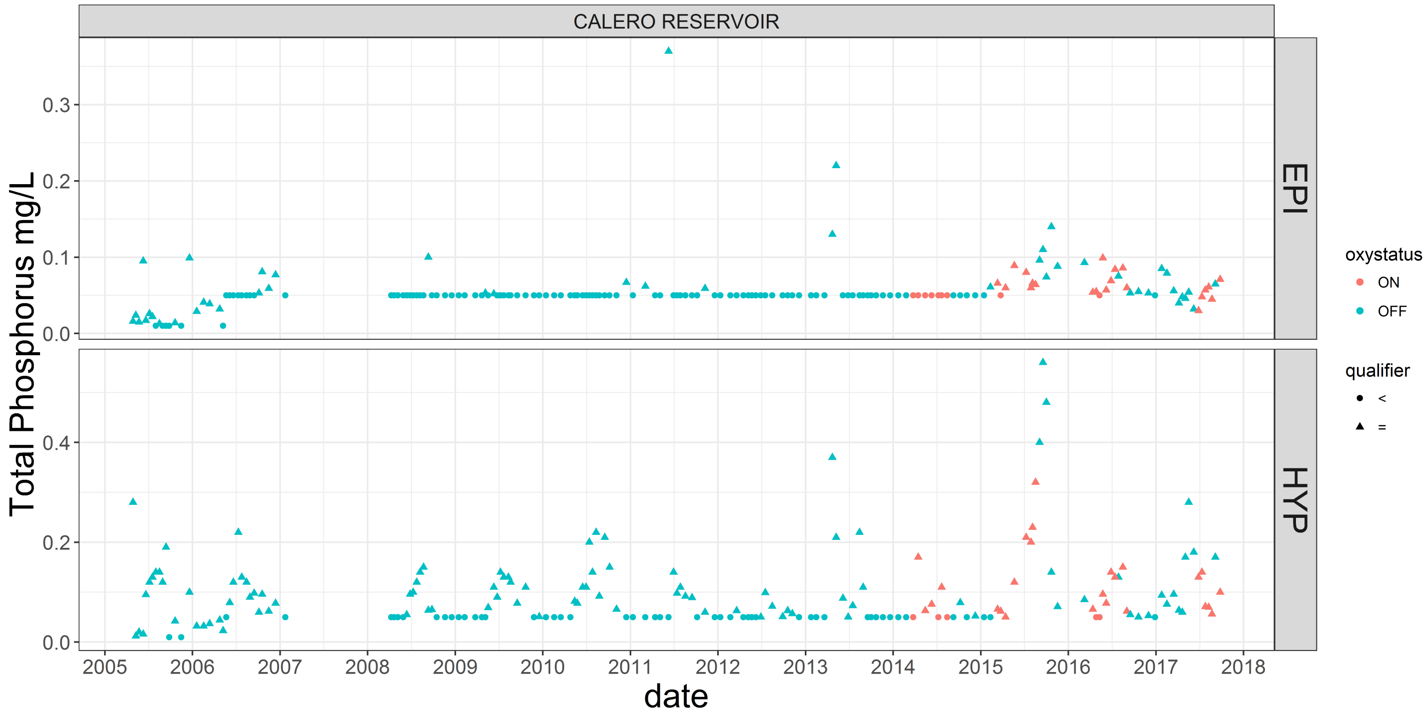
CALERO RESERVOIR
Total Phosphorus mg/L
EPI
HYP
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0.1
0.2
0.3
0.4
2005
2006
2007
2008
2009
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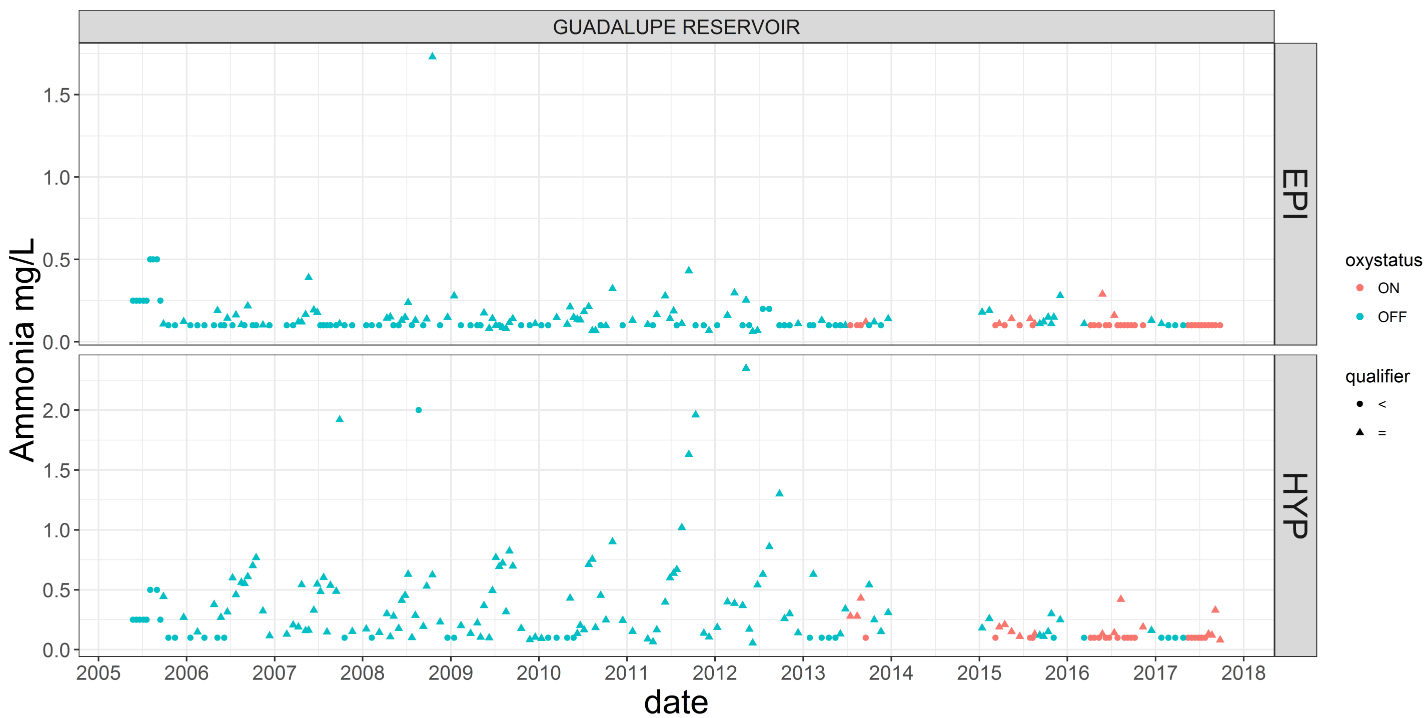
2011
2012
2013
2014
2015
2016
2017
2018
date
oxystatus
ON
OFF
qualifier
<
=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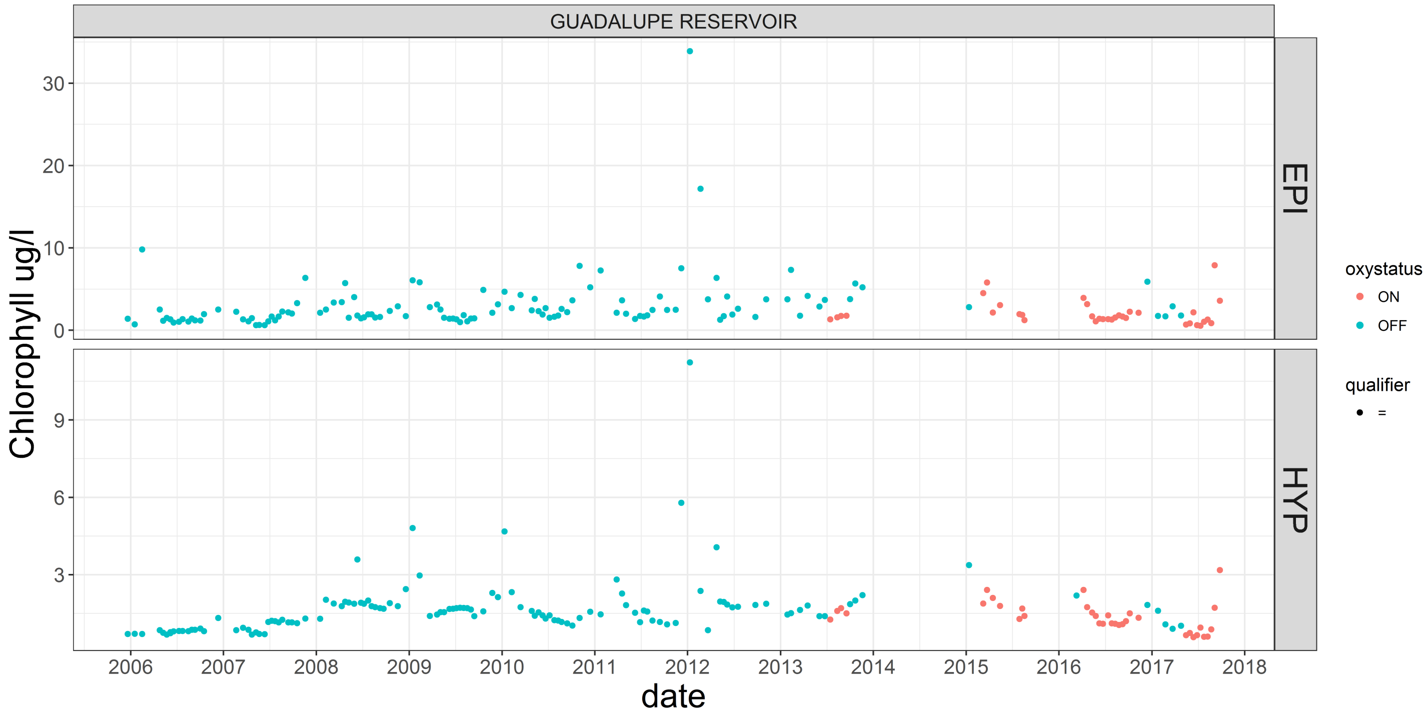
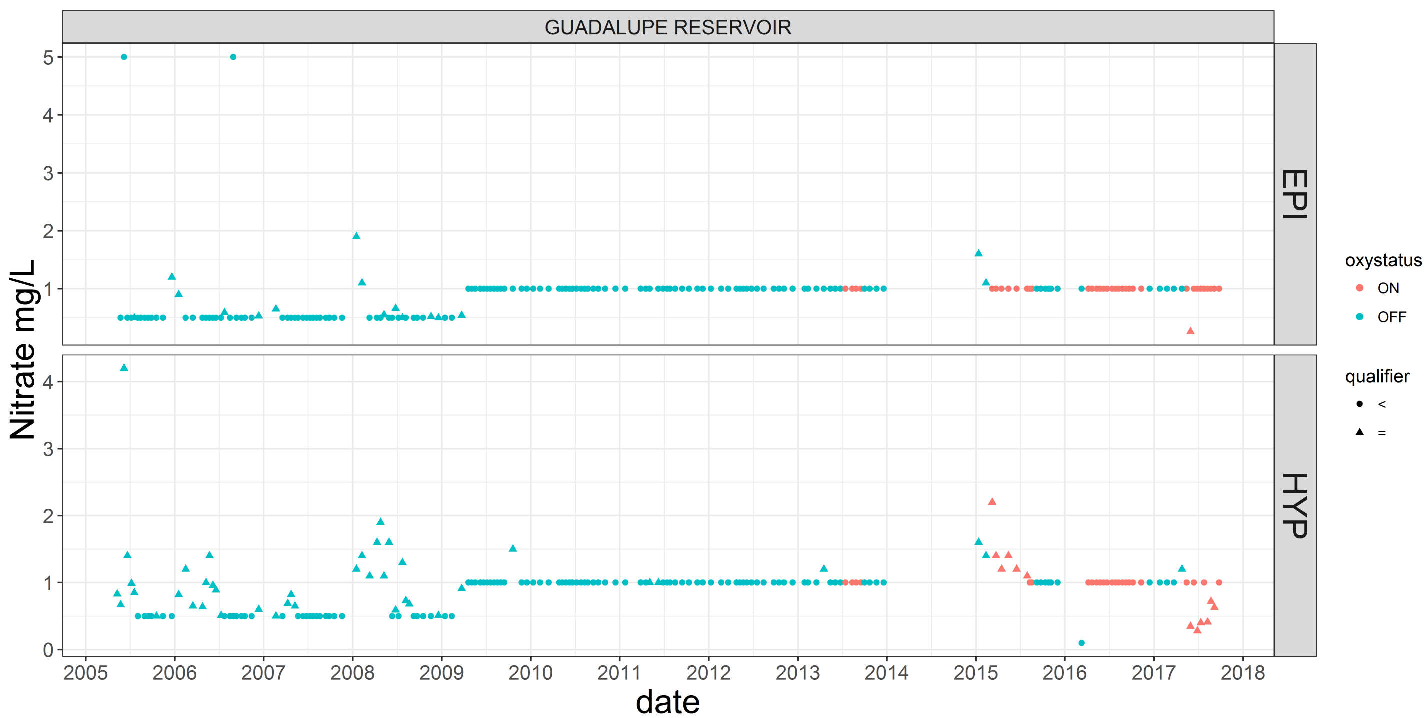
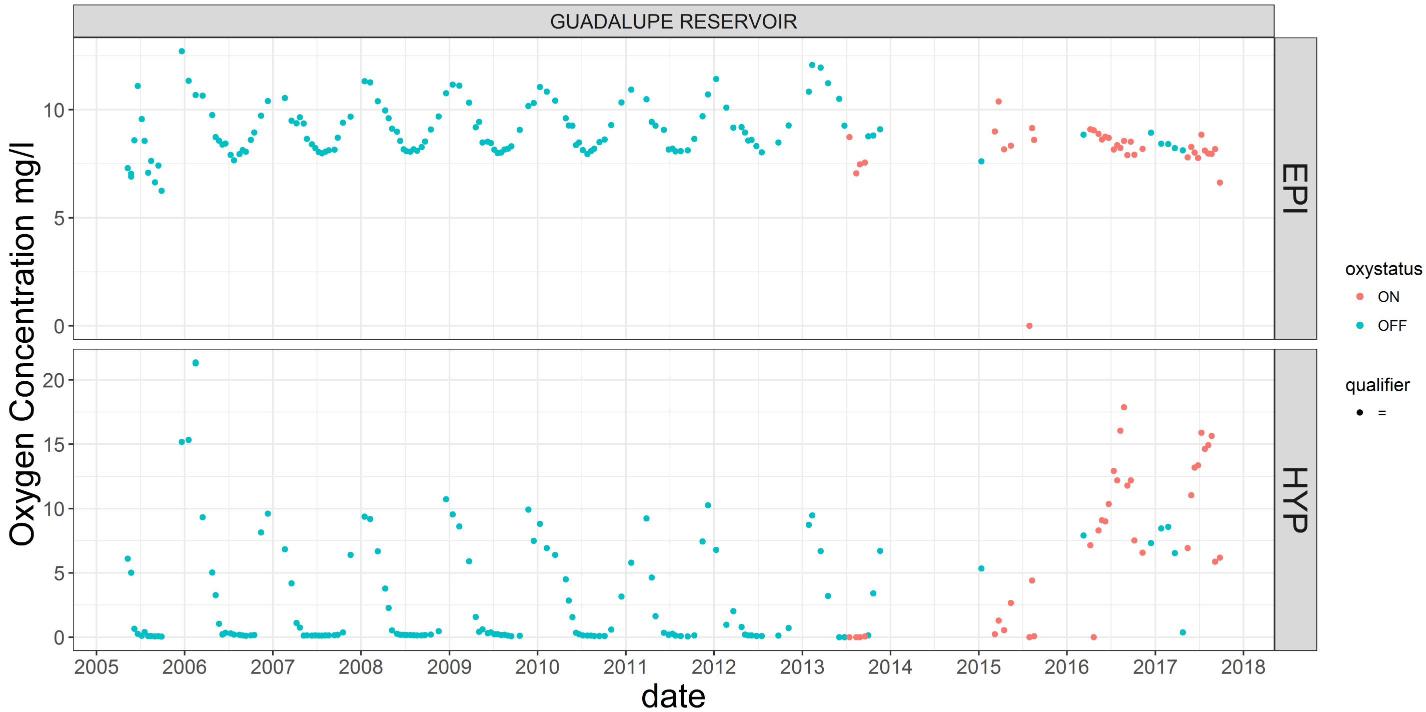
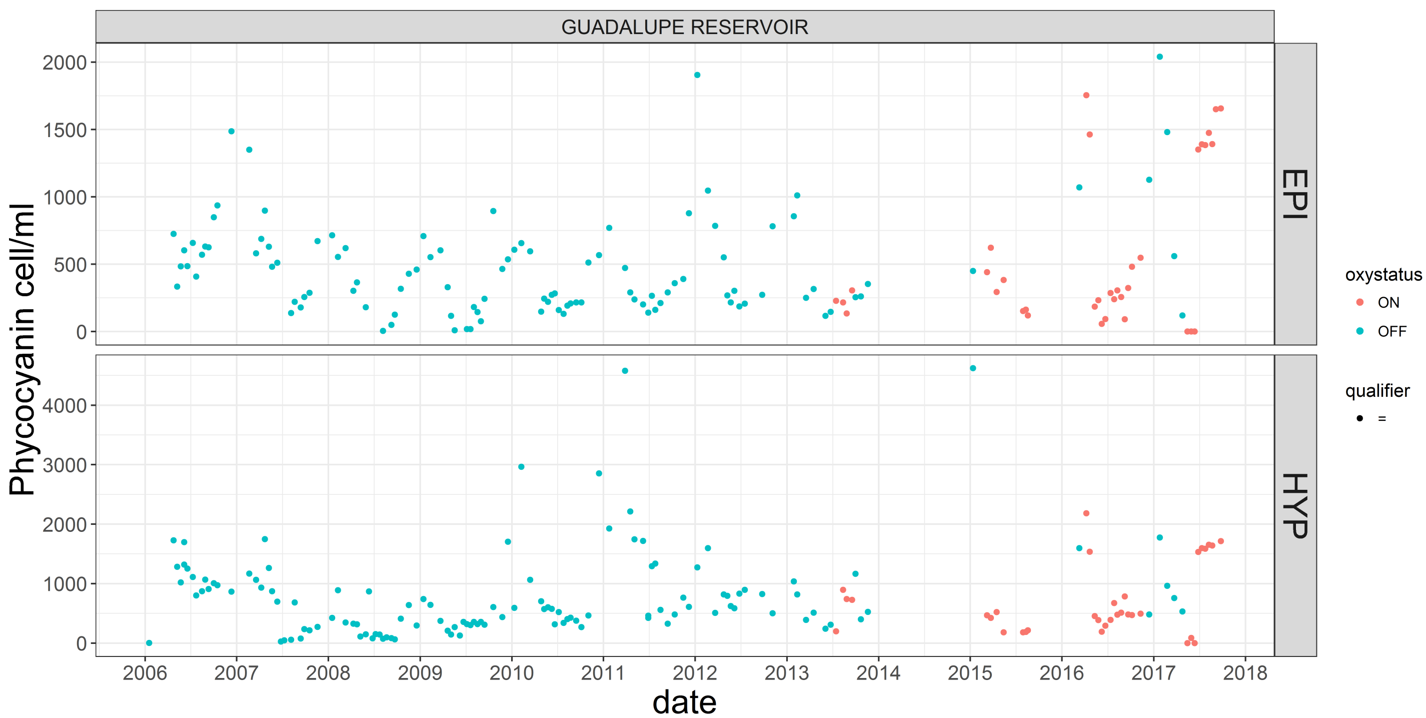
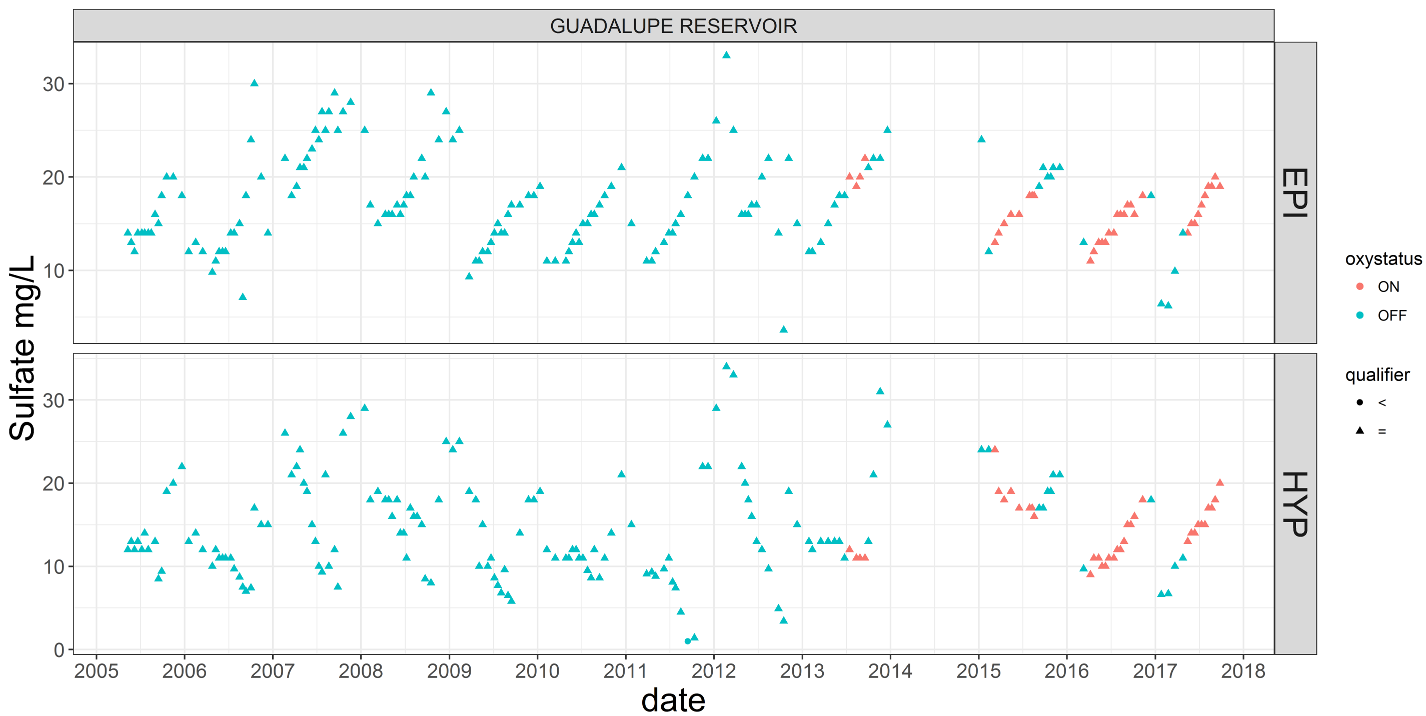
Ammonia mg/L
EPI
HYP
0.0
0.5
1.0
1.5
2.0
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
date
oxystatus
ON
OFF
qualifier
<
=

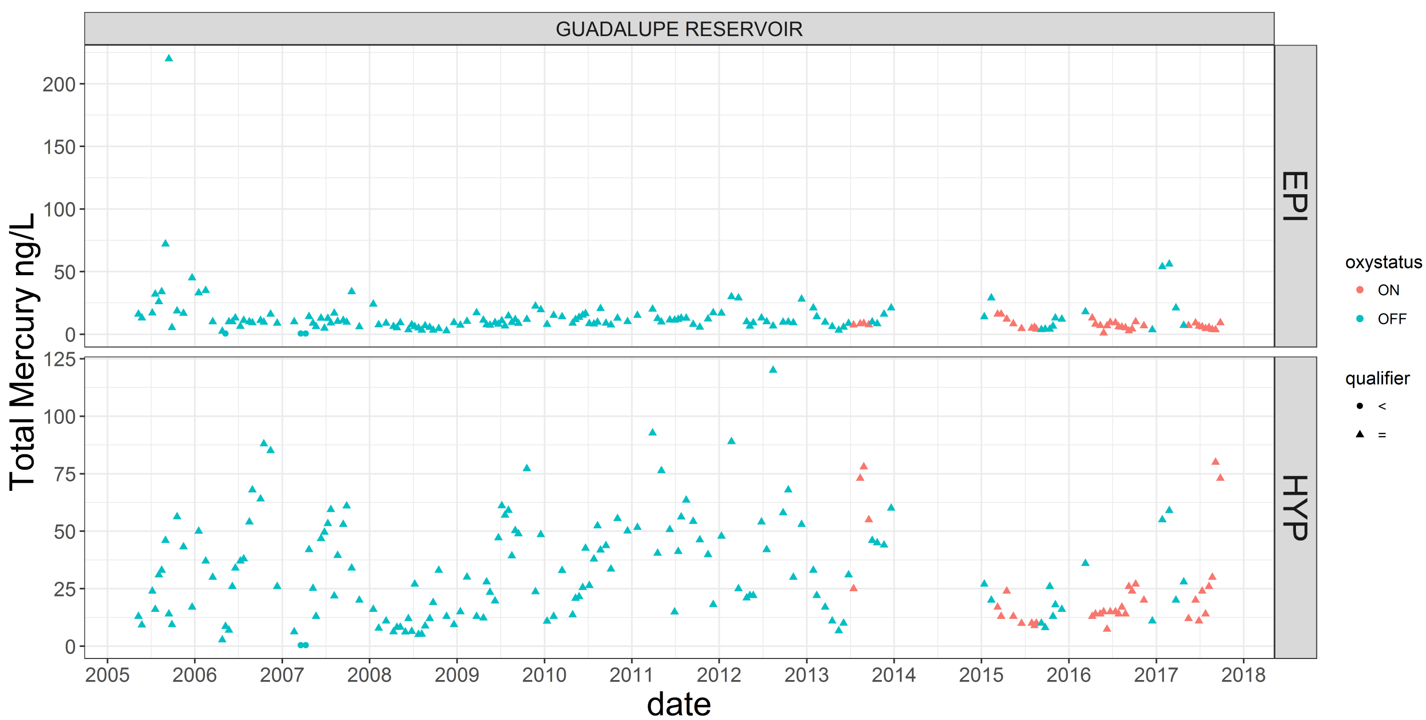

GUADALUPE RESERVOIR
Chlorophyll ug/l
date
EPI
HYP
oxystatus
ON
OFF
qualifier
=

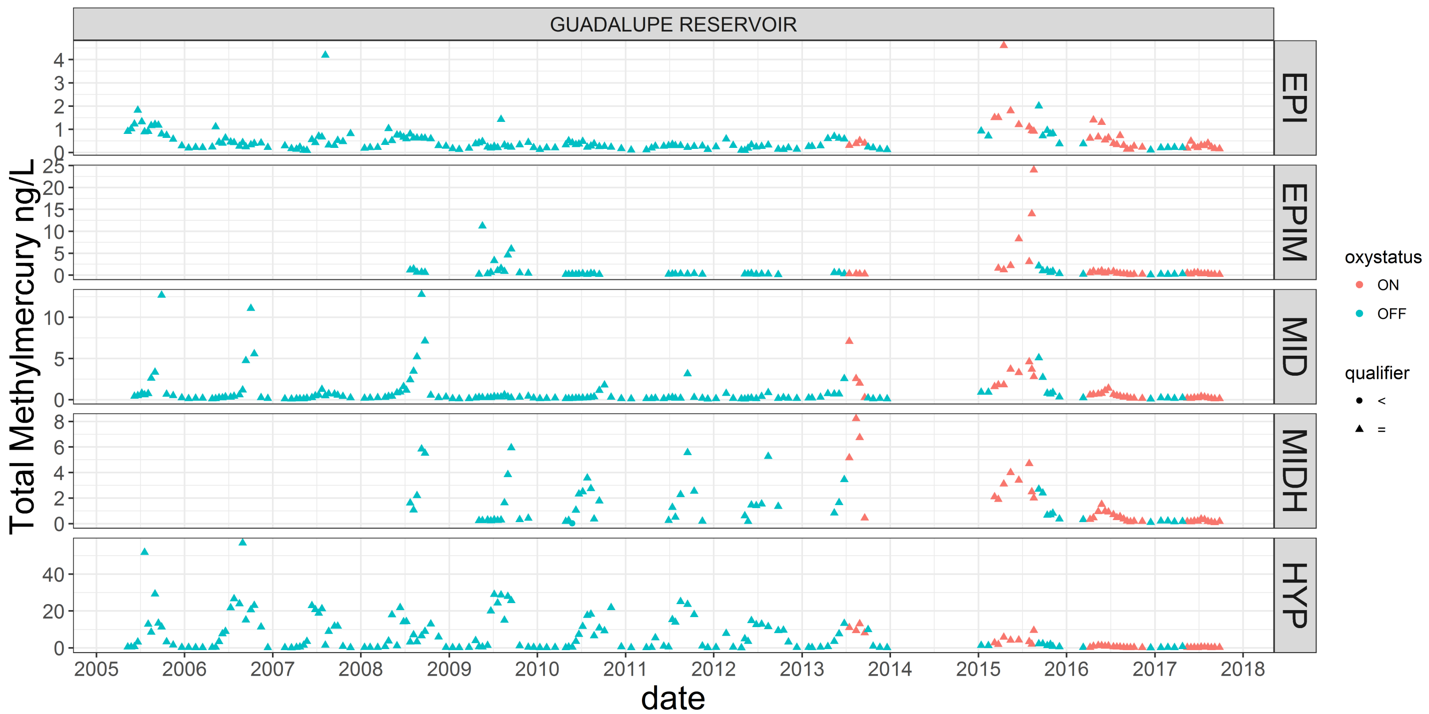

GUADALUPE RESERVOIR
Nitrate mg/L
date
EPI
HYP
oxystatus
ON
OFF
qualifier
<
=

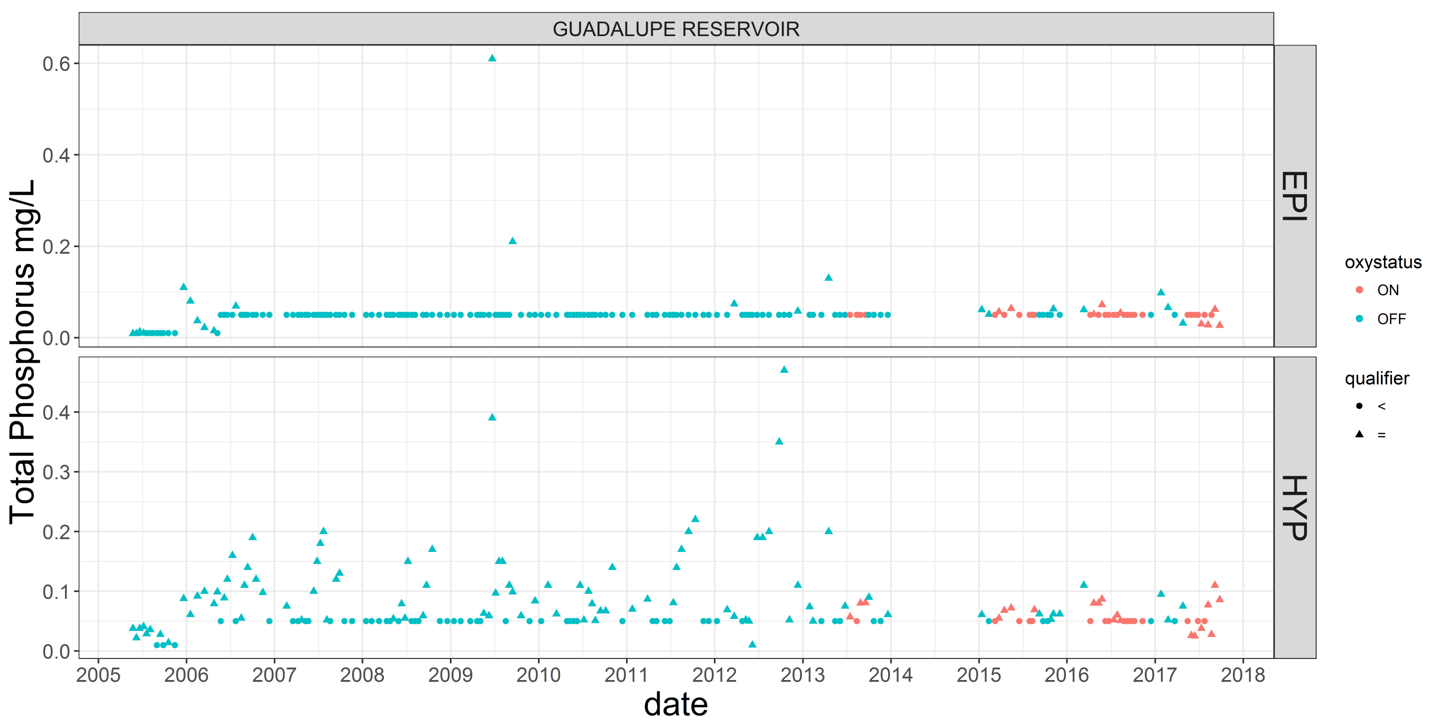

GUADALUPE RESERVOIR
Oxygen Concentration mg/l
EPI
HYP
date
oxystatus
ON
OFF
qualifier
=

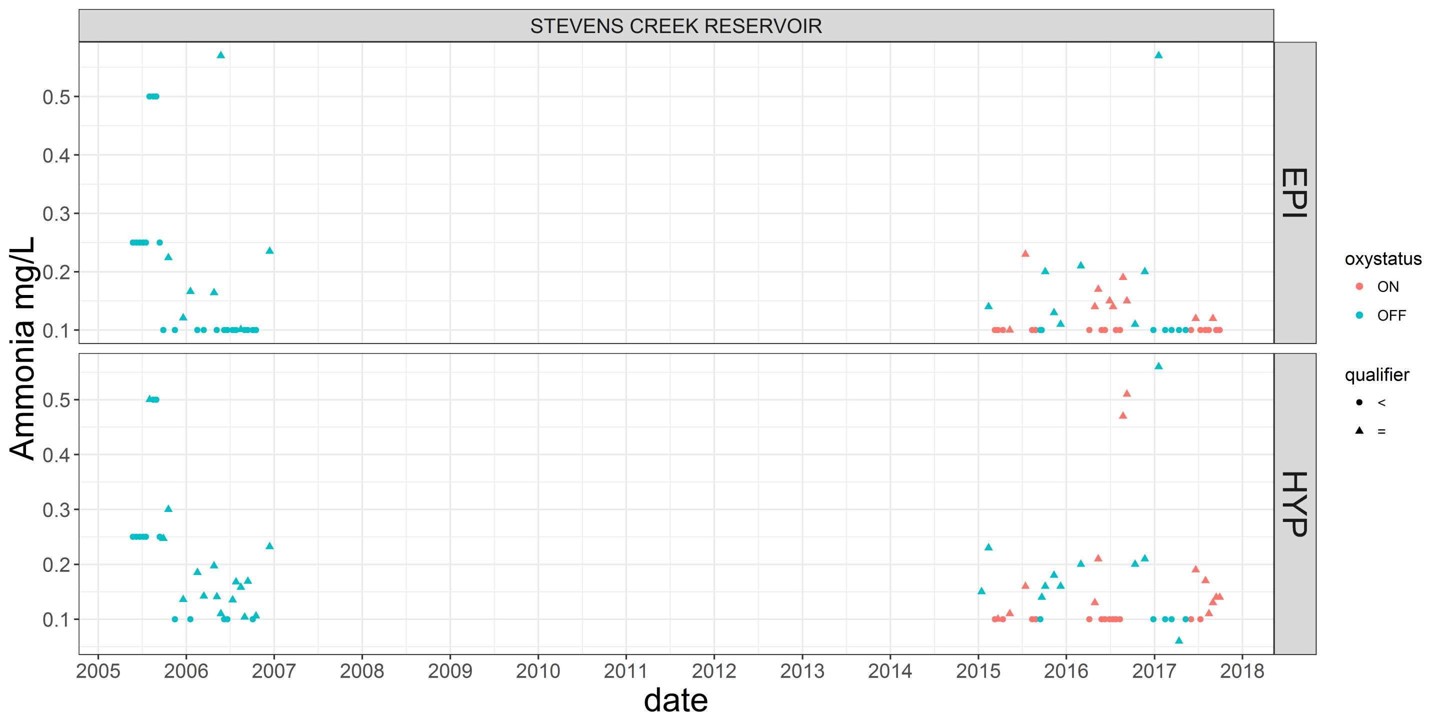

GUADALUPE RESERVOIR
Phycocyanin cell/ml
EPI
HYP
date
oxystatus
ON
OFF
qualifier
=

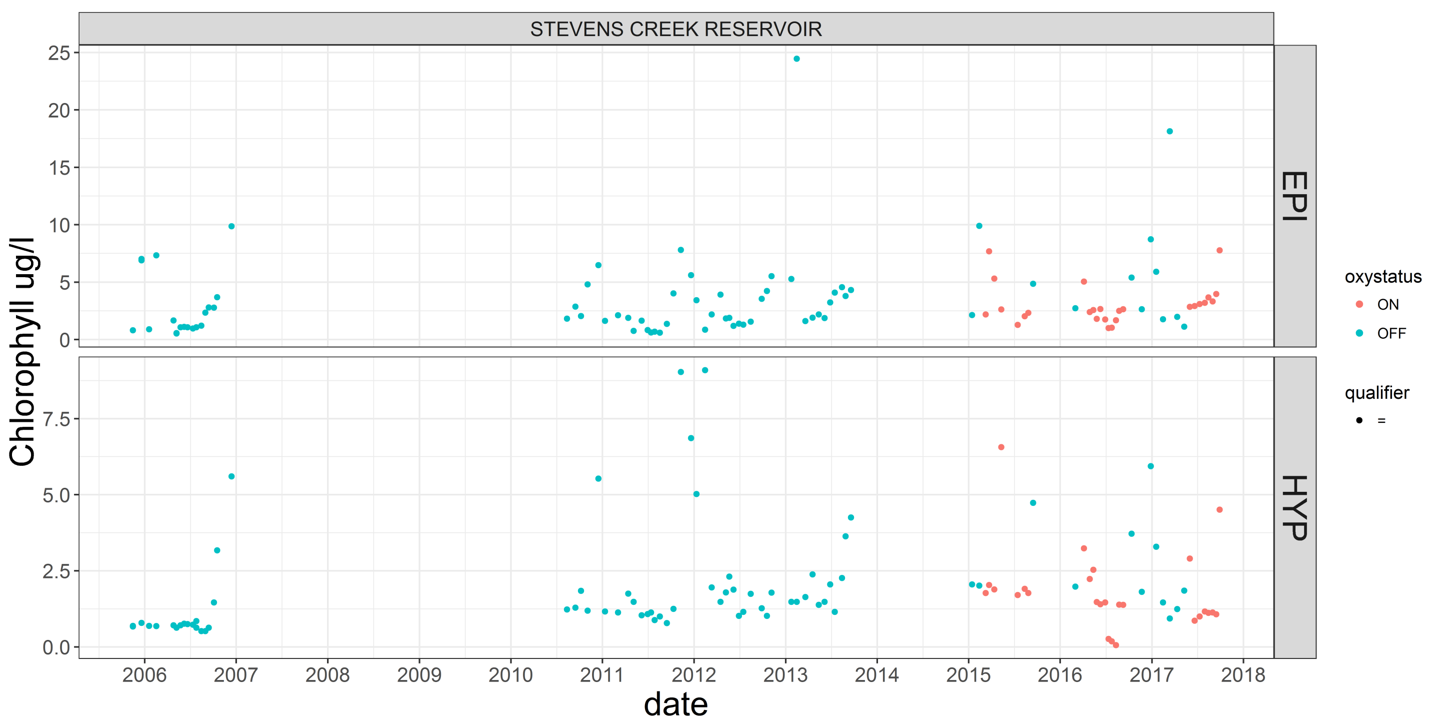

GUADALUPE RESERVOIR
Sulfate mg/L
EPI
HYP
date
oxystatus
ON
OFF
qualifier
<
=
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018

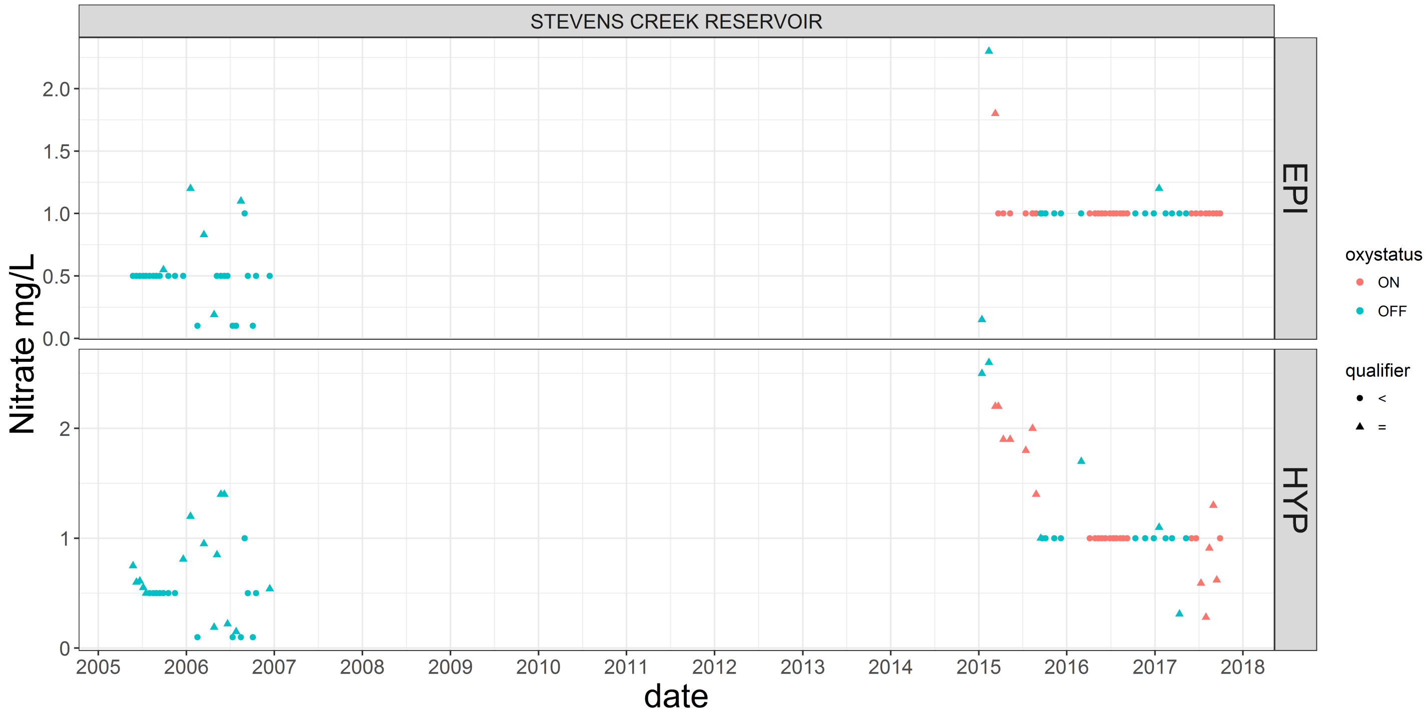

GUADALUPE RESERVOIR
Total Mercury ng/L
EPI
HYP
date
oxystatus
ON
OFF
qualifier
<
=
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018

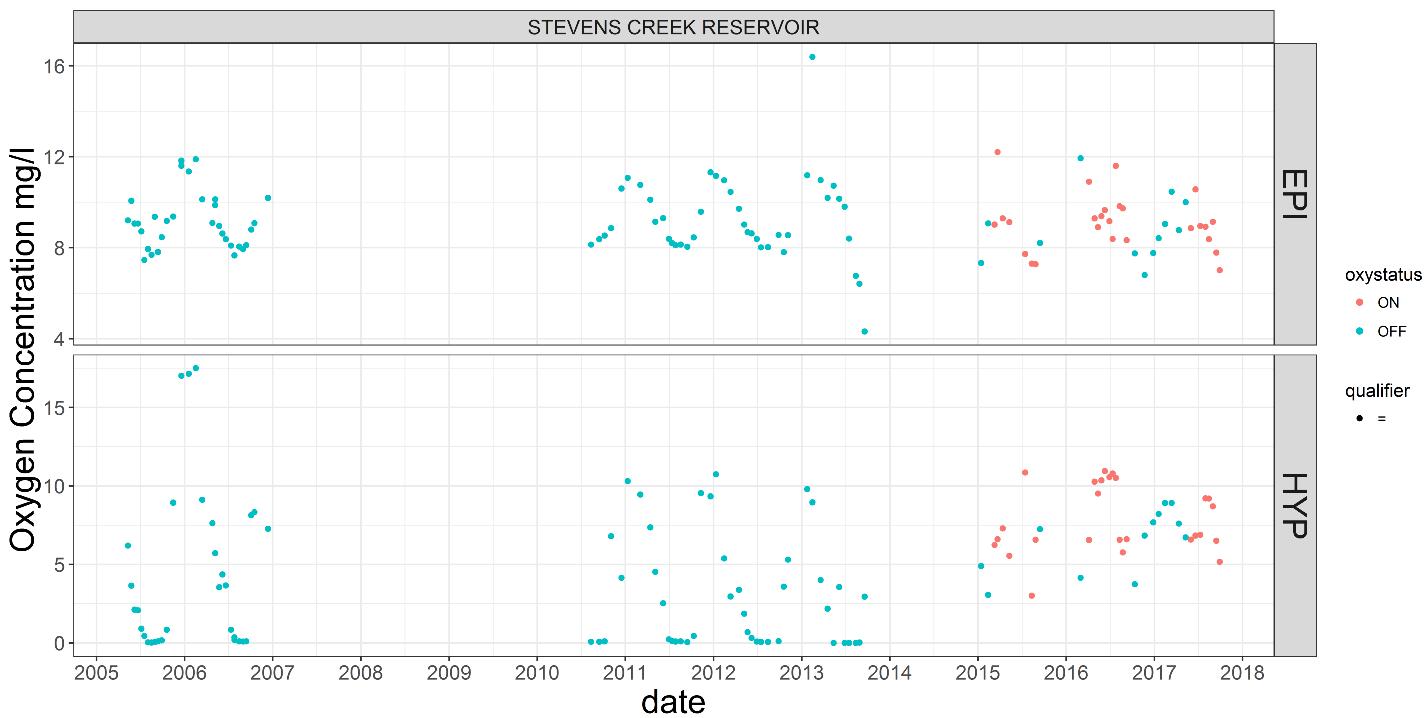

GUADALUPE RESERVOIR
Total Methylmercury ng/L
EPI
EPIM
MID
MIDH
HYP
date
oxystatus
ON
OFF
qualifier
<
=

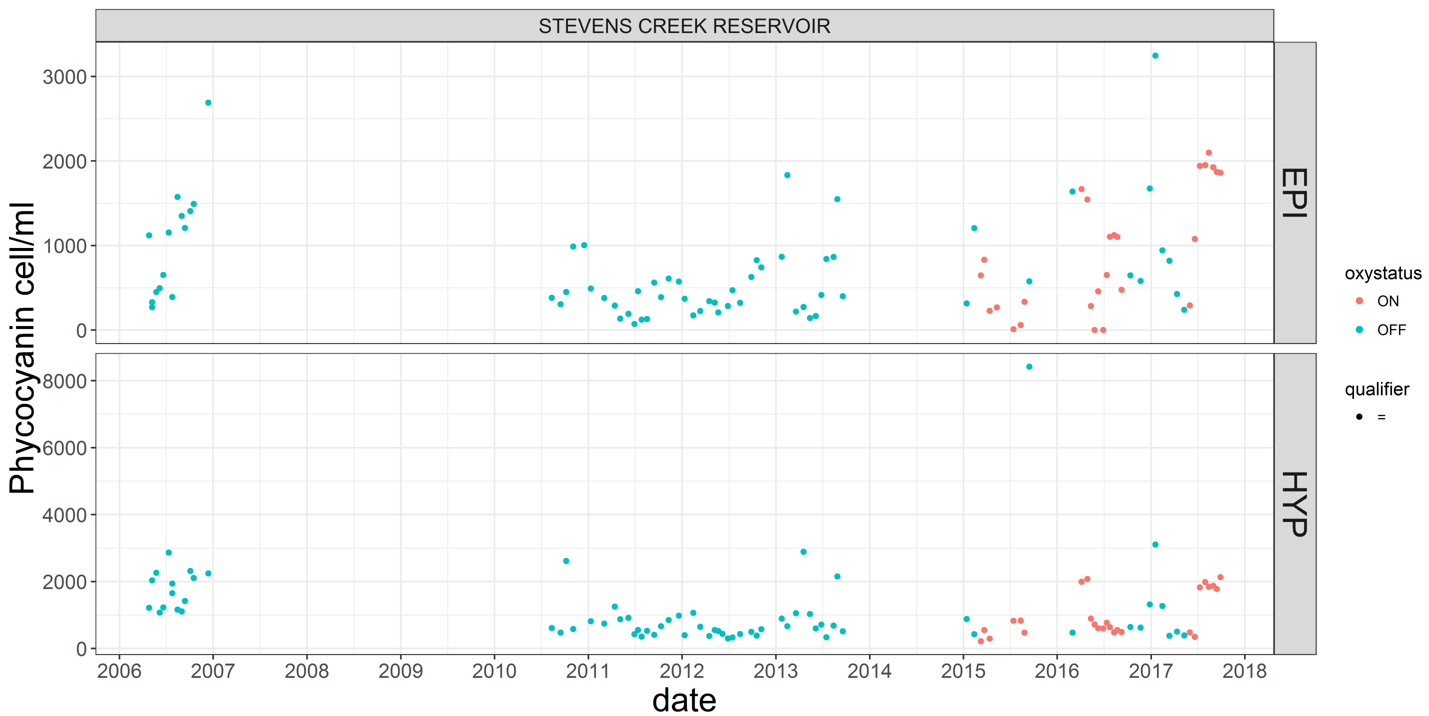

GUADALUPE RESERVOIR
Total Phosphorus mg/L
EPI
HYP
date
oxystatus
ON
OFF
qualifier
<
=

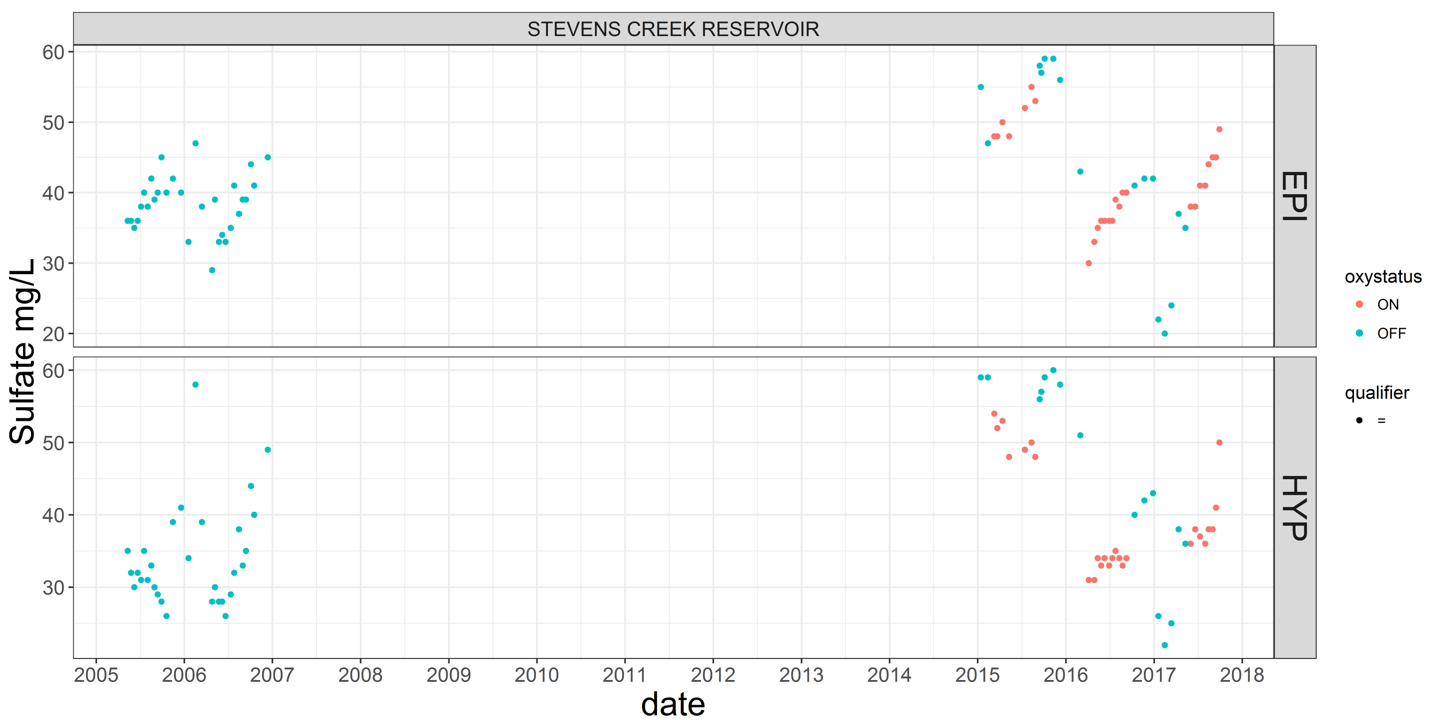

STEVENS CREEK RESERVOIR
Ammonia mg/L
EPI
HYP
date
oxystatus
ON
OFF
qualifier
<
=

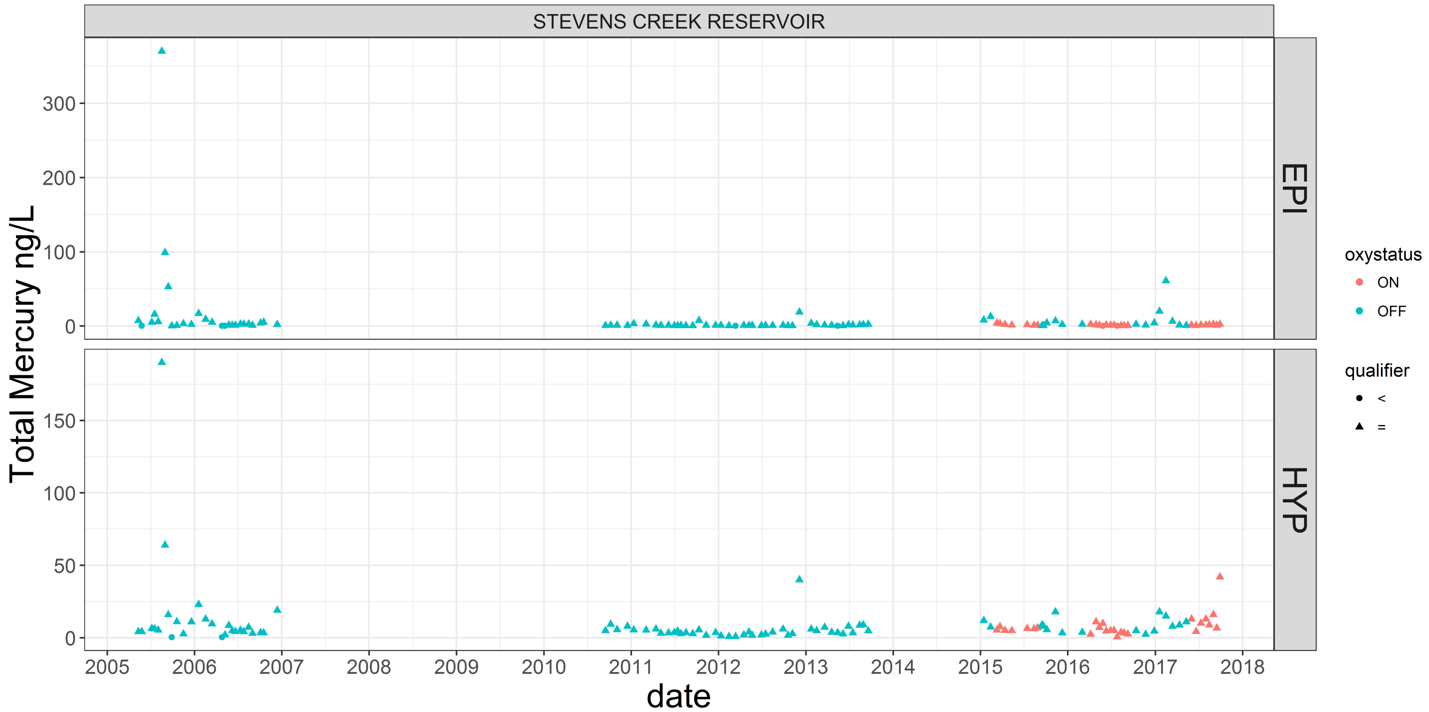

STEVENS CREEK RESERVOIR
Chlorophyll ug/l
EPI
HYP
date
oxystatus
ON
OFF
qualifier
=


STEVENS CREEK RESERVOIR
Nitrate mg/L
EPI
HYP
date
oxystatus
ON
OFF
qualifier
<
=

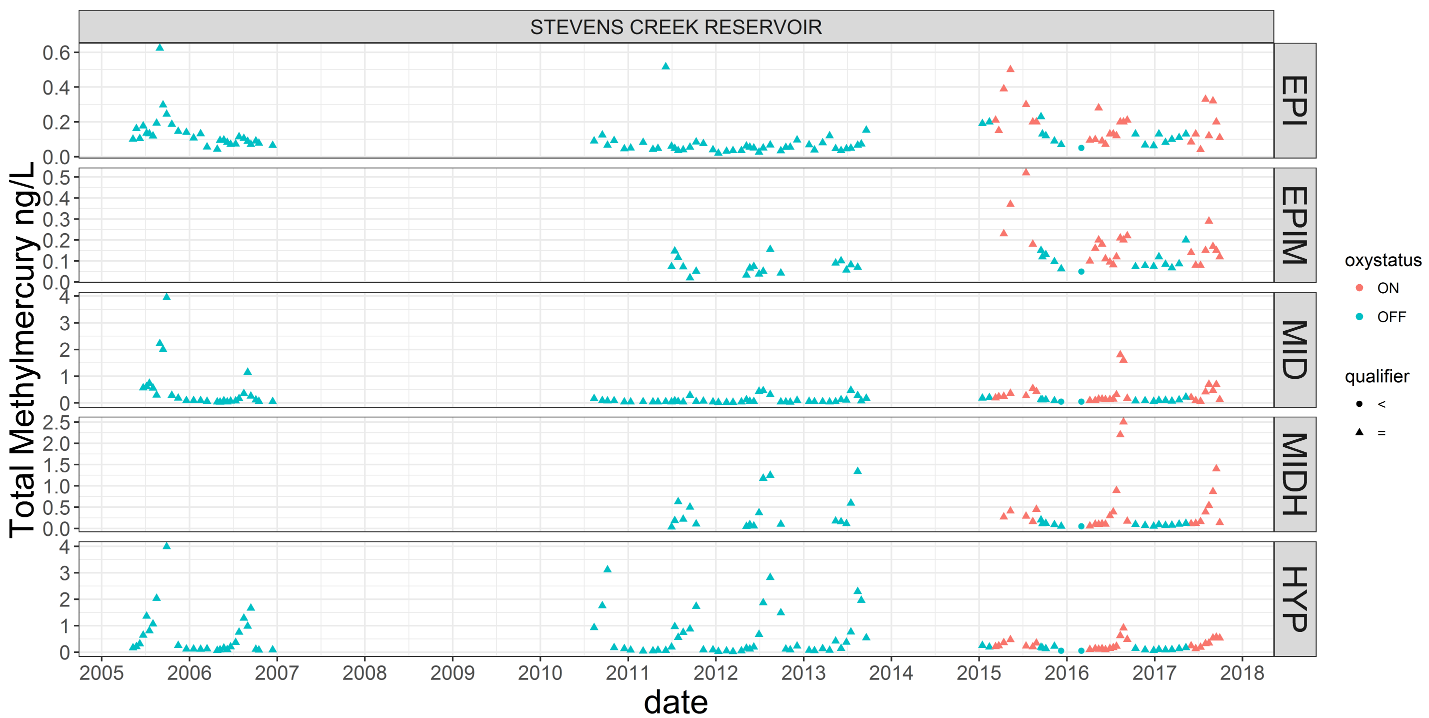

STEVENS CREEK RESERVOIR
Oxygen Concentration mg/l
EPI
HYP
date
oxystatus
ON
OFF
qualifier
=


STEVENS CREEK RESERVOIR
Phycocyanin cell/ml
EPI
HYP
date
oxystatus
ON
OFF
qualifier
=

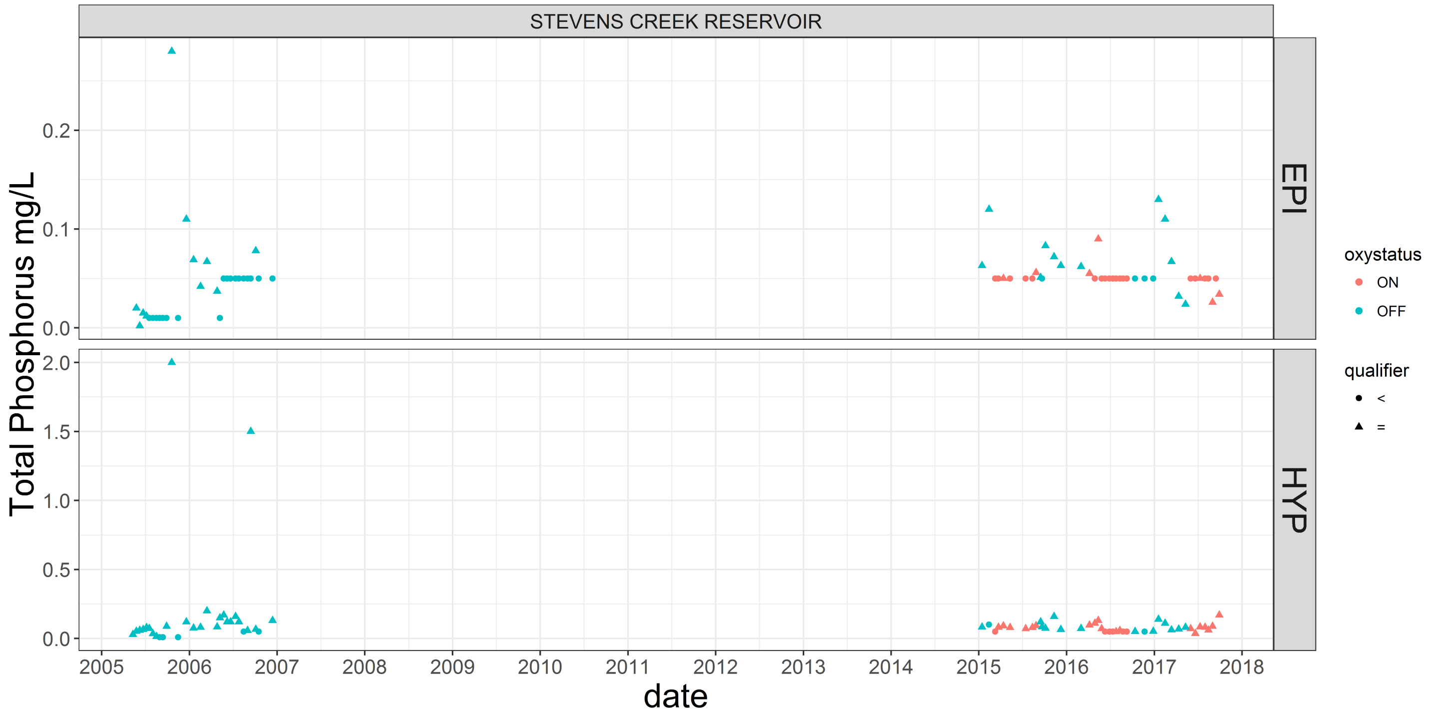

STEVENS CREEK RESERVOIR
Sulfate mg/L
EPI
HYP
date
oxystatus
ON
OFF
qualifier
=



STEVENS CREEK RESERVOIR
Total Mercury ng/L
date
EPI
HYP
oxystatus
ON
OFF
qualifier
<
=



STEVENS CREEK RESERVOIR
Total Methylmercury ng/L
date
EPI
EPIM
MID
MIDH
HYP
oxystatus
ON
OFF
qualifier
<
=


STEVENS CREEK RESERVOIR
Total Phosphorus mg/L
0.0
0.1
0.2
0.0
0.5
1.0
1.5
2.0
EPI
HYP
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
date
oxystatus
ON
OFF
qualifier
<
=